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ABSTRACT

This student's manual for the medical laboratory student is one of a series of self-contained, individualized instructional materials for students enrolled in training within the allied health field. It is intended to provide study materials and learning activities that are general enough for all medical laboratory students to use to enhance their understanding and performance at the training station. The material should be used under coordinator or instructor supervision. Seven units cover these topics: care of supplies and work area, collection and transportation of specimens, urinalysis, hematology, serology, bacteriology, and chemistry. Each unit follows this format: objectives, a list of new terms and definitions, and information to aid the learner in mastering the objectives. (YLB)

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Medical Laboratory Assistant

Student's Manual

By Sara Barnett

Published by

Center for Occupational Curriculum Development
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The University of Texas at Austin

CE 032 953

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Foreword

This publication is one of the new series of self-contained instructional materials for students enrolled in training within the allied health field. Students using this material can work at their own pace, learning those things that best fit their specific needs. The design of the material makes it possible to relate experiences on the job to information studied at school.

Although the material is individualized, it should be used under the supervision of a coordinator or instructor. For that reason, two basic publications are offered—a student's manual and an instructor's guide.

The *student's manual* is written for the person in training. It includes objectives, and information to aid the learner in mastering the objectives.

The *instructor's guide* provides the competencies progress chart and answers to the learning activities, unit tests, and final test. In addition to the answers, copies of the learning activities, unit tests, and final test are provided. A sample coordinating work sheet is also provided to match related instruction with job experiences for each student.

Although the manual and the guide are copyrighted, you should feel free to reproduce copies of the activities, unit tests, and final test. If you prefer, these same sheets can be ordered separately for individual students from the Center for Occupational Curriculum Development, P. O. Box 7218, The University of Texas at Austin, Austin, Texas 78712.

Jack E. Robertson, Director
Center for Occupational
Curriculum Development

Preface

There is a need for current and relevant information to assist the medical laboratory student with individual classroom study relating directly to the profession of medical technology. Realizing the enormous diversity of equipment, personnel, facilities, and physician and patient demands, I have tried to provide study materials and learning activities that are general enough for all medical laboratory students to use to enhance their understanding and performance at the training station. The use of trade names is for clarification and example only and is in no way intended as an endorsement of the product.

I would like to thank the following persons for their assistance, contributions, and words of encouragement:

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Last, but not least, my appreciation is expressed to the Texarkana Independent School District, with its fine administrators, faculty, and students, and to Wadley Hospital for all their cooperation in this endeavor.

Sara Barnett

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UNIT 1

Care of Supplies and Work Area

Objectives: After completing this unit, you will be able to–

1. clean and maintain the work area and equipment;
2. clean the area and equipment aseptically;
3. log incoming and outgoing specimens;
4. maintain files of laboratory records or reports; and
5. organize the storage area for equipment, supplies, and reagents.

New Terms and Definitions

aseptic–free from disease-causing germs.
aspiration–to draw fluid from a body cavity.
autoclave–apparatus for sterilization by steam under pressure.
biopsy–to take out a small piece of tissue for microscopic examination.
contaminated–unsterile or unclean as a result of direct contact or exposure to germs.
disinfectant–an agent that kills bacteria.
infectious–capable of causing infection or disease.
materiél–equipment, apparatus, and supplies used by an institution.
reagent–chemical substance.
resection–to cut off a portion of a bone or other body structure.
sterilization–to make free of all living germs.
warranty–a written guarantee of the maker's responsibility for a product, including repair or replacement of defective parts.

Clean and Maintain Work Area and Equipment

The cleaning and maintenance of the work area of the laboratory is usually the responsibility of the medical laboratory worker. Work tables or benches must be kept free of clutter. Spills should be wiped up at once. The entire work area must be dusted or cleaned with a damp cloth at the end of each work day.

If you work in a small clinic or a doctor's office, you may be responsible for keeping the reception room neat and attractive. Patients will be more comfortable and less apprehensive in pleasant surroundings. The housekeeping department or janitorial service is probably responsible for heavy cleaning, such as the general cleaning of floors, walls, and windows. However, the medical laboratory worker should check to be sure that the room is adequately cleaned. He or she may be responsible for keeping the room neat as patients come and go during the day. Papers, drink bottles, cans, or cups must be removed. Ashtrays must be emptied and cleaned. (Some waiting rooms have "No Smoking" signs. Others do not have ashtrays in sight. Thus, the patient must ask for an ashtray if he or she wishes to smoke.) Odors that may be disagreeable to patients should be carefully controlled. Room fresheners or sprays may help. It is sometimes hard for the medical laboratory worker to remember that odors to which she or he has become accustomed may be obnoxious to patients. The

temperature and ventilation should be kept at a comfortable level.

Various types of surfaces found in the service area require different types of cleaning. *Chrome* and *stainless steel* should be wiped clean with a damp cloth and polished with a dry cloth. A nonscratching cleanser followed by polish will remove most stains.

Enamel surfaces are cleaned by wiping with a damp cloth and polishing with a dry cloth. Light waxing at intervals helps to maintain the surface. Chipped places may be repaired with enamel finish.

Wood surfaces should be dusted daily with a soft cloth and waxed or polished frequently. This is usually done by the housekeeping department or janitorial service.

Plastic surfaces, as well as glass or ceramic tile, are cleaned with a mild soap or detergent, rinsed with water, and dried with a soft cloth.

Clean Area and Equipment Aseptically

The above cleaning procedures are adequate for general cleaning. If the work area and equipment require aseptic cleaning, then different materials must be used. *Aseptic cleaning* means that you are ridding the area to be cleaned from disease-causing germs. To clean tabletops aseptically, they should be washed with a disinfectant. A *disinfectant* is a chemical that kills harmful bacteria.

Contaminated glassware is placed in porcelain or stainless steel containers with snug-fitting tops until it can be completely sterilized. Pipettes may be placed in cylindrical jars or rectangular pans containing disinfectant. The contaminated glassware is then autoclaved to ensure the destruction of harmful bacteria.

Contaminated specimen cartons, applicators, tongue depressors, cotton, and other combustible material should be discarded into a garbage pail with a tight lid. The pail should be lined with a disposable bag so that the entire contents of the garbage can be removed and placed directly into the incinerator. As few workers as possible should handle contaminated materials.

All infectious materials, such as sputum from tuberculous patients, must be autoclaved before discarding.

Common Methods of Sterilization

The commonly used methods of sterilization of contaminated equipment are chemical, hot air (dry heat), and autoclaving.

Chemical sterilization is used to sterilize items that would be destroyed by heat, such as polyethylene items, lenses, or electrical equipment. Items are sterilized by exposure to ethylene oxide for 3 to 5 hours. Articles must be aired after chemical sterilization to avoid the hazard of toxic residue.

Dry heat may be used to sterilize glassware or metal instruments. A temperature of 160°C for 2 hours is usually used.

The *autoclave* sterilizes by the use of steam under pressure. It is used to sterilize glassware, sharp-edged or hinged instruments, syringes, needles, rubber catheters, drains, or linens.

The choice of sterilization methods depends on the type of material to be decontaminated and the equipment available in your laboratory.

In some instances, disinfection is adequate for decontamination. Some items may be disinfected by placing them in boiling water for 10 minutes. Commonly used disinfectants are isopropyl or ethyl alcohol in 70% concentration. There are several commercially prepared disinfectants. They must be used in the precise concentration and for the exact time specified by the manufacturer.

The means and methods of sterilization and disinfection may vary depending on the needs of the individual institution. Even so, the work area, equipment used, and contaminated specimens must all be decontaminated and made safe to protect workers and patients.

Types of Specimens Received

Many types of specimens are received in the medical laboratory. These include any body excretion such as urine, feces, or sputum. Other specimens may be obtained by needle aspira-

tion, such as blood, spinal fluid, pleural fluid, and body cells from the liver, sternum, or kidney. Tissue obtained from resections or from biopsies is sent to the laboratory for examination.

Another type of laboratory specimen is a *smear*. This is obtained by touching an area with a cotton swab, then smearing the swab on a microscope slide. Such material is most commonly obtained from the nose, throat, or a wound. Many other types of specimens are received in the laboratory, depending on the capability of the laboratory and the need of the patient.

Logging Incoming and Outgoing Specimens

Most laboratories have an established routine for receiving specimens. It is necessary to have a definite area where specimens may be placed when they arrive in the laboratory. It may be one of the duties of the medical laboratory worker to identify incoming specimens and route them to the correct work area.

In every laboratory, some method of logging or recording specimens must be devised. The method depends on the type of institution, the type of specimen, and the number of specimens handled.

In very large laboratories, *numbering systems* and *color coding* are used. The color coding indicates the various divisions of the laboratory. This makes it easier to route specimens quickly to the correct division. Numbers may be used to identify the specimen, the accompanying information, and all the tests required to complete the laboratory examination. A permanent record of the test may be filed using the same number. There are many types of numbering devices on the market, such as numbering machines or numbered adhesive tapes.

In smaller laboratories where there are fewer specimens to handle, simpler logging methods may be used. A *request form* with a single carbon copy may be satisfactory. This form indicates the type of test ordered and all the information needed to identify the patient, including the patient's chart number. This number is affixed to the specimen. When testing is complete, the original request, with test results, is returned to the patient's chart, using the number to ensure identification. The copy is filed in the laboratory.

In very small laboratories, specimens may be numbered with a marking pencil, and the same number recorded on the accompanying request sheet. Whatever method of logging is used, mistakes in identification of specimens are intolerable. All logging methods should be aimed at elimination of error.

Every means possible should be used to identify a specimen with incomplete information. Keep in mind that no laboratory specimens are unimportant. Some are extremely difficult to collect, and, indeed, it is impossible to replace most laboratory specimens. In any case, if it is impossible to identify a specimen correctly, it must be discarded.

Packaging Outgoing Specimens

Few laboratories are equipped to conduct every laboratory test that may be required. Some specimens must be sent to other laboratories. Outgoing specimens must be packaged in correct or suitable containers. They must be correctly labeled. The label should include all pertinent information. This includes the patient's name, address, and chart number; the date and hour of collection; the type of specimen; the exact tests ordered; and the name and address of the attending physician. This same information should be filed in the sending laboratory to ensure identification of the specimen, to verify having sent the specimen, and for patient billing.

Maintain Files of Laboratory Records or Reports

Copies of the results of every laboratory test performed are kept in the medical facility. These copies are used to determine charges for laboratory services, to replace reports that may

have been lost or misplaced, and to provide information and statistics for research.

Modern electronic equipment has made it possible to keep large numbers of laboratory records and reports in a small space. Even smaller medical facilities frequently use microfilm to store laboratory reports. In some laboratories, however, medical laboratory workers may be required to hand-file laboratory reports. Reports may be filed under the patient's name, patient's number, type of test, or even the name of the attending physician. Whatever method is used, filing should be an easy task. It must be arranged so that anyone can use the filing system and quickly retrieve the information needed.

Organize Storage Areas

Every medical facility must store supplies or materiéls. The methods used depend on the needs of the individual institution. Most hospitals have a large storage area. Supplies are ordered in quantity and dispensed as needed. Usually a written request is required. It is the responsibility of the materiéls department to maintain an inventory of these supplies and order additional items as they are needed.

In smaller offices, the medical laboratory worker may be assigned the task of maintaining supplies. A list of all equipment and supplies must be prepared. Regular inventory will tell what has been used and what must be ordered. A pad and pencil located in the supply area to record needs as they occur will make it easier to remember items needed. A list of suppliers with names and telephone numbers or sales representatives should be prepared and kept up to date.

An accurate file of operating manuals, warranties, and service personnel for all equipment should be prepared and maintained. One factor to consider in the purchase of equipment is the availability of maintenance service.

All supplies should be kept where they are easily accessible, yet protected from damage. The storage of chemicals requires special planning.

Here are some tips to remember when *storing chemicals:*

1. Chemical supplies should be stored in a cool, dark place—preferably in a cabinet behind closed doors. Sunlight causes deterioration of most chemicals.
2. Do not store strong acids next to bases.
3. Never use metal lids on the containers of acids and bases because metal will corrode.
4. Poisons should be stored separately from other products routinely used in the laboratory. They should be labeled clearly as *poison.* Most poisons are packaged with a distinctive cap to make identification easier.
5. Flammable materials must be stored away from heat.
6. Narcotic drugs must be stored in a locked cabinet that is out of sight, or at least inconspicuous (not easily noticed).
7. Every bottle must have a legible label. It is best to keep the original label, but replacement labels must be an accurate, identical copy of the original.

Some logical method of glassware storage must be devised in the laboratory for both efficiency in use of the equipment and to avoid breakage. Glassware must be easy to find, whether it is stored by size, type, use, or a combination of all three. Pipettes and test tubes are usually stored in drawers built to hold specific sizes. Other glassware is stored on accessible shelves. Open glassware that is not used often should be stoppered to keep dust out.

Large pieces of equipment should be stored out of the way. That which is most frequently used should be most accessible. The lavish use of labels helps to identify materiéls quickly. Such labels may indicate not only the name of the equipment, but also what test it is used for.

In storing equipment and supplies, it is important to choose a method that will be most efficient for your particular laboratory and one that can be easily followed by all employees using the equipment.

UNIT 2

Collection and Transport of Specimens

Objectives: After completing this unit, you will be able to–

1. collect blood samples;
2. collect fecal specimens;
3. collect urine specimens;
4. prepare specimens for testing; and
5. prepare specimens for shipment.

New Terms and Definitions

ambulatory–capable of walking; not bedridden.

anticoagulant–substance that prevents blood from clotting (coagulation).

blood plasma–the liquid part of the blood that has been prevented from coagulating.

blood serum–the clear liquid part of the blood after coagulation.

catheter–a tube put in the body to permit injection or withdrawal of fluids.

distal–farthest from the center.

hemolysis–the rupture of red blood cells with the release of hemoglobin into the plasma or serum.

lancet–a sharp-pointed, two-edged surgical knife.

rapport–a relation marked by harmony.

residual–left over.

supernatant–the clear liquid remaining at the top after the heavier parts settle to the bottom.

tourniquet–device for stopping bleeding by compressing a blood vessel.

venipuncture–puncture of a vein, usually for withdrawing blood.

Collection of Blood Samples

Blood samples are used for many laboratory investigations. If more than a few drops are needed, blood is collected by **venipuncture.** It is very important to establish rapport with the patient before you begin this procedure. You will need to identify yourself and tell the patient why you are there. You must carefully identify the patient, use the name tag and bed tag, and call the patient by name. Review the doctor's orders to make sure you are collecting the correct amount of blood and that you have prepared the correct containers to receive the specimen.

You should appear calm, reassuring, and sympathetic to the patient. Remember that, although you may collect many blood specimens every day, the procedure is probably new and frightening to the patient.

You should place the patient in a comfortable position. Ambulatory patients should be seated so there is a place to support the arm. Bed patients will remain in bed.

It is wise to briefly explain the procedure for venipuncture to the patient, but avoid going

into detail about the tests to be performed. Your information may confuse the patient. The doctor will explain the tests to the patient. You should explain to the patient that there is little discomfort and no residual effect from venipuncture.

Venous blood is usually taken from veins in the inner bend of the elbow (fig. 1). If for some reason the elbow cannot be used, or if you have difficulty getting blood from these veins, other sites may be used. These include the lower arm, wrist, back of hand, fingers, lower leg, ankle, or upper surface of the foot. However, these veins are usually very small although they may appear large. They have a tendency to roll, and they may have toughened walls. Therefore, the veins in the elbow are almost always most satisfactory for venipuncture.

You will need to assemble your equipment before you collect blood. If you are *using a syringe,* you will need sponges, a tourniquet, a syringe with a needle, test tubes, and slides if required.

Next, you will select the site for puncture. Place the tourniquet above the site, and tie it with a slipknot so it can be removed with one hand. Ask the patient to open and close the hand, causing the veins to become congested. Then choose a vein that is clearly evident and seems so well supported by tissue that it will not roll.

Cleanse the area with a sponge and antiseptic such as alcohol or soap solution. Dry with a sterile sponge, or air-dry.

Insert the needle cleanly and rapidly with the bevel upward. Pull back on the plunger. As blood appears in the barrel of the syringe, you are assured that the needle is in the vein. Then release the tourniquet, and collect the amount of blood required.

Place a sponge over the puncture site, but do not apply pressure until the needle is withdrawn. Then apply pressure firmly, and flex the arm until bleeding stops. Caution the patient that a bruise will result if the wound is allowed to bleed.

Make blood smears if required. Then remove the needle from the syringe, and carefully distribute blood to the indicated containers. Do not force blood through the needle, nor handle roughly, because this may cause the red blood cells to rupture with resulting hemolysis. Label all tubes and slides. Check to be sure the patient is all right, then dismiss the patient or leave the room. Be sure to take all of your equipment with you.

Fig. 1 Taking venous blood.

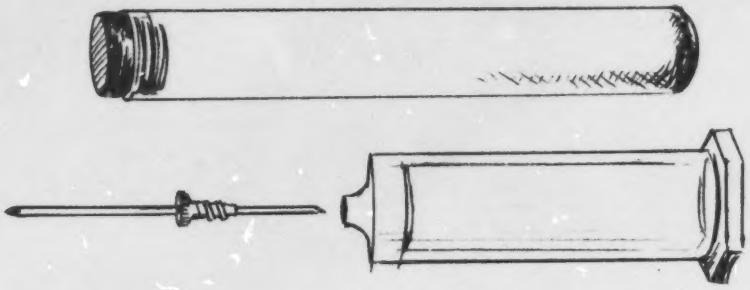


Fig. 2. Equipment for collecting blood

If you are *using a vacuum tube* to collect blood, the procedure is similar. You will assemble sponges, a tourniquet, vacuum tube, and needle. Screw the needle into place on the adapter (holder).

Place the tube loosely into the holder contacting the double-pointed needle, but not puncturing the stopper.

Follow the procedure used for collecting blood with a syringe to prepare the patient and insert needle.

After the needle end is correctly inserted into the vein, push the stoppered tube firmly onto the adapter point of the needle, puncturing the stopper. Continue to aspirate until the tube fills to the predetermined amount. Vacuum tubes will not fill completely.

If *multiple samples* are needed, remove the first tube, and rapidly replace it with a second, third, etc. Be very careful to keep the needle in place inside the vein as you change tubes.

When all specimens have been collected, remove the needle, and care for the patient as described earlier. Label all tubes carefully. Then you may dismiss the patient. Always thank patients for their cooperation, and leave with a friendly attitude.

After blood specimens have been collected, you must be sure that they are properly labeled. The label should include the patient's name and number, the doctor's name, the date, and the test required. It may include the name of the collector, the patient's room number, and the time of collection, or any pertinent information.

For some tests, *uncoagulated blood* is required. An anticoagulant is a substance that prevents clotting of blood. Since various anticoagulants are available, be sure to select the correct one for the test to be done.

After blood has been collected and tubed, it must be handled carefully until it reaches the laboratory. Blood placed in a test tube containing an anticoagulant must be inverted gently to mix.

If serum is needed for a laboratory test, *whole blood* is collected in a plain tube. It should not be inverted or mixed. Mixing may cause hemolysis of the serum. You should allow the blood to clot for 30 minutes at room temperature. Then carefully dislodge the clot from the tube wall by moving a clean applicator stick around the test tube. Decant or pipette the serum into a clean test tube, and label.

Blood placed in a test tube containing thrombin or SST is inverted and can be centrifuged after 5 minutes.

Caution must be taken to *prevent hemolysis* of blood. The following tips will be useful:

1. Use a sharp, smooth needle of appropriate size, usually 20 or 22 gauge.
2. Enter the vein as directly as possible.
3. Do not tie the tourniquet too tightly, and release it before aspirating blood.
4. Use only a dry syringe and tubes.
5. Remove the needle before distributing blood to tubes.
6. When an anticoagulant is used, mix blood gently by inverting stoppered tube. Don't shake the tube.

7. If serum is needed, allow clot to form before centrifuging.

Capillary blood is collected when a few drops are enough for testing. Usually the specimen is collected from the ring finger or middle finger. The earlobe may be used if fingers are thick-skinned or very rough, or if the patient will be handling contaminated materials. The toe is used if neither a finger nor an earlobe is available. In infants, either the toe or the heel is used.

The first step in **collecting capillary blood** is the same as that for venipuncture. Establish rapport with the patient, and identify yourself. Identify the patient by means of name tag and bed tag and by calling him or her by name. Review the doctor's orders.

Then you are ready to assemble the needed equipment. You will need sponges, a sterile lancet, microtubes and/or blood pipettes, slides, diluting fluid if needed, and adhesive bandage.

Next, select the site to puncture. Never use an area that is swollen, scarred, calloused, or bruised. Cleanse the area with a disinfectant sponge, and wipe with a dry sponge. (If the site is wet, the blood will spread out instead of forming a drop.)

Grasp the finger firmly across the middle or first joint (fig. 3). The patient may involuntarily move if you do not hold the finger securely. Quickly puncture the skin with a sterile, disposable lancet. Wipe off the first drop of blood. To take advantage of gravity, keep the patient's hand conveniently low. Using gentle pressure on the finger, fill containers as ordered. Do not allow air bubbles to form in collection tubes or pipettes. Collect with a continuous flow of blood. Hold up the distal end of the collection tubes. Then seal the ends of capillary tubes with clay.

Finally, prepare blood smears on microscope slides if requested. Then cleanse the finger with a disinfectant sponge, and apply pressure with a dry sponge to stop bleeding.

When the procedure is complete, be sure the patient is all right. Thank the patient for cooperating, and then dismiss her or him. If the

Fig. 3. Collecting capillary blood

patient is in a hospital, be sure to collect all your equipment and the specimens before you leave the room.

Collection of Fecal Specimens

Laboratory tests on feces are important in diagnosis. The patient should be carefully instructed in the method of collection to ensure a satisfactory specimen.

Hospital patients are given a clean, dry bedpan for defecation. Nursing personnel will collect the specimen and send it to the laboratory.

If the patient is to collect the specimen, then he or she must fully understand *what* is to be collected, *how* it is to be collected, and *what* container should be used. The following general instructions will be helpful:

1. The specimen must not be contaminated with urine.
2. No laxative should be taken unless it is ordered by the doctor.
3. A wide-mouthed container with a tight-fitting lid and a few wooden tongue blades should be provided.
4. The patient may defecate in a clean, dry bedpan or on a clean paper plate.
5. The patient will collect a small amount of feces (size of a pecan), place it in the container, and cover securely. The patient must take care to avoid contaminating the outside of the container.
6. Finally, the patient must wash hands, attach completed label, and return the specimen to the laboratory.

The information on the label should include the patient's name, the time and date of collection, the name of the doctor, and any other pertinent information.

Fecal material collected by the doctor during rectal examination is sometimes sent to the laboratory for study.

A variety of tests may be performed on fecal specimens. Some of these require very specific instructions for collection. Details such as preservatives, special containers, exact time of collection, or a constant temperature may be important. Such instructions should be made very clear to the patient. Some examples of types of tests are these:

1. Gross examination for consistency, color, mucus, or blood.
2. Occult blood.
3. Detection of ova and parasites.
4. Viral and bacterial cultures.
5. Detection of ameba; trophozoites and cysts.

Collection of Urine Specimens

Perhaps the most common laboratory procedure is the urinalysis, which is a series of tests done on urine.

A routine urinalysis means the series of urine tests selected by a given institution to be performed on most urine specimens. Usually, a routine urinalysis includes a gross examination of appearance, reaction, simple chemical tests, and a microscopic examination of sediment. A *routine specimen* means a urine specimen collected at any time during the 24-hour day. Such a specimen will vary in concentration depending on the patient's liquid intake. Sometimes the first urine specimen collected in the morning is preferred because it will be more concentrated. A routine urine specimen is almost always collected upon admission to the hospital or when visiting the doctor's office.

For some tests, a *24-hour* urine specimen is needed. This means that the entire quantity of urine voided for 24 hours is collected and sent to the laboratory for testing.

Sometimes, a *midstream or "clean catch"* urine specimen is preferred. This means the specimen is essentially free of contamination. The patient is instructed to clean the urethral area if female, or glans if male. (In the hospital, nursing personnel will assist the patient.) The patient then begins to void into the toilet. This rinses the urethra. The midstream urine is collected in a clean container. The remaining urine is voided into the toilet. If the midstream specimen is to be used for bacteriologic studies, a sterile container must be used.

Rarely, catheterization is used to obtain clean urine specimens for bacteriologic study. Such procedures are infrequently ordered

because the use of the catheter may cause urinary tract infections.

In the hospital, nursing service is responsible for the collection, labeling, and test requests for urinalyses. Specimens should be delivered to the laboratory promptly. You may be responsible for logging the specimens and assigning numbers after they have reached the laboratory.

In a clinic or doctor's office, the medical laboratory worker may be responsible for urine specimen collection. The following procedure may be used:

1. Provide a clean, dry container.
2. Ask the patient to provide a urine specimen.
3. Tell the patient the location of the rest room and where the collected specimen is to be placed.
4. When the specimen is returned, label it properly, and provide the proper report form.
5. Perform the tests as soon as possible. If there must be a delay, refrigerate the urine specimens. Bacteria multiply rapidly, and leucocytes and casts tend to disintegrate at room temperature.

Urine specimen containers must be clean and dry. If they are to be used for bacteriologic examination, they must be sterile. Most hospitals use plastic, disposable containers. Plastic, glass, or metal containers are commonly used in smaller laboratories because they may be washed and reused. Occasionally, a patient may be asked to collect a urine specimen at home. It is advisable to provide the patient with a specimen container. This way you can be sure the container is clean and uncontaminated.

Preparation of Specimens for Testing

The medical laboratory worker may be asked to *process specimens.* This means the preparation of specimens for actual analysis after they have been collected.

The various tests done on blood require different preparations. Whole blood should be kept in the original stoppered container until it is ready for analysis. If there is a delay of more than an hour between collection and testing, then the specimen should be stored in the refrigerator.

Some blood tests are done using serum. Serum is the liquid part of blood that remains when a clot forms. To process a blood specimen for serum collection, you first allow the blood to clot. Then, rim the tube with an applicator to loosen the clot. Next, centrifuge the specimen, and collect the supernatant serum. Serum may be frozen if analysis is delayed more than 4 hours.

If an anticoagulant is added to whole blood, the liquid part of the blood in which the cells are suspended is the plasma. To collect plasma, you must centrifuge the blood. The cells will then collect at the bottom of the tube. The supernatant fluid is plasma. Plasma should be refrigerated if there must be a delay between processing and analysis. Plasma may be frozen if the delay is longer than 4 hours.

One problem that you must avoid in processing blood specimens is hemolysis. *Hemolysis* means the rupture of red blood cells with the release of hemoglobin into the plasma or serum.

Blood collected soon after a patient has eaten a meal may contain neutral fats. This will cause the serum or plasma to have a milky appearance and may cause error in test results. To avoid this problem, be sure to instruct the patient to fast before the blood is collected.

Other materials for laboratory testing, such as urine, feces, or bacterial studies, may require special processing, but the general rule is to examine the specimens as quickly as possible after collection. If prompt analysis is impossible, then use the appropriate means to preserve the specimen.

Preparation of Specimens for Shipment

Specimens may be sent to laboratories outside your medical facility for various reasons. A primary concern is cost. It is less expensive for some medical facilities to send laboratory work

to a laboratory that does a large volume of work than to maintain a complete laboratory with trained personnel for their small number of tests.

Another reason is convenience. Often routine laboratory tests are performed on site while the patient is waiting. Tests other than routine are sent to outside laboratories. By concentrating on routine tests, laboratory personnel will have time to handle these tests for the normal flow of patients.

Individual preference is another reason for sending specimens to an outside laboratory. Some physicians, patients, and insurance companies prefer to have tests performed in a laboratory other than the facility in which the specimen is collected.

Finally, many complex laboratory tests are difficult to perform with reproducible results. Therefore, the expertise and equipment required is a reason for sending specimens to another laboratory. The more complex tests that are infrequently ordered are sent to laboratories where large numbers of such tests are done. These laboratories have the time, space, equipment, and personnel to perform such tests.

Before you can prepare the specimens for shipment, you must know what kind of specimens are needed for the exact tests requested. Requisition slips must be carefully prepared noting all relevant information. Examples of information needed are patient's name, address, phone number, age, sex; the sending clinic or hospital; chart or room number; the doctor's name and address; a description of the specimen; and tests ordered.

Some specimens need special preparation before they are sent out. Plasma or serum may be separated from whole blood. If a preservative or anticoagulant is used, be sure it is the correct one. Some specimens even require special temperatures or sprays. The special preparations ensure that the specimen will arrive safely and intact.

Specimens must be mailed in appropriate containers following specific instructions. For example, frozen specimens are packed in dry ice. Acute and convalescent samples are sent at the same time. Of course, these must be very carefully labeled to distinguish them. Containers used should prevent spillage or breakage. Addresses must be clearly typed or printed. Some laboratories will provide containers for mailing specimens.

Commercially prepared containers designed to protect contents are available. These can be obtained in a variety of shapes and sizes to fit almost any specimen, such as test tubes, urine containers, or slides.

A variety of specimens may be sent to laboratories outside your medical facility. Included are whole blood, blood serum, or plasma; smears of blood or bacteria; Papanicolaou smears; specimens of urine, feces, or sputum; or tissue from biopsy or resection.

There are also a variety of laboratories that might receive such specimens. They may be sent to city, county, state, or federal public health laboratories. There are many research facilities and medical schools where specimens may be sent. Insurance company laboratories sometimes require that specimens be sent to their facility. Many private laboratories are equipped to perform complex laboratory tests.

The decision whether to perform a given test at your medical facility or to mail it to another laboratory depends on many factors. Whatever choice is made, the primary deciding factor should be to make available to patients the best possible laboratory service at the lowest possible cost.

UNIT 3

Urinalysis

Objectives: After completing this unit, you will be able to–

1. give directions for collection of urine specimens;
2. label urine specimens;
3. perform macroscopic examinations, and record physical characteristics;
4. perform specific gravity determinations;
5. interpret "stix" and record readings of–
   a. pH
   b. protein
   c. glucose
   d. ketones
   e. bilirubin
   f. urobilinogen
   g. blood
   h. nitrite
6. examine urine specimens microscopically; and
7. perform pregnancy test on urine.

New Terms and Definitions

agglutination–clumping or adhesion of particles.

amorphous–without a definite shape.

anuria–failure of kidneys to function; absence of excretion of urine from the body.

artifact–an artificial structure.

casts–cylindrical structures with blunt ends and parallel sides and composed of mucoprotein.

convoluted–rolled together or coiled.

decant–pour off liquid so sediment remains in bottom of container.

ectopic pregnancy–implantation of fetus outside uterine cavity.

hydatidiform mole–mole formed by rapid multiplication of chorionic villi to form a mass of cysts resembling a bunch of grapes; causes false positive pregnancy test.

macroscopic–visible to the unaided (naked) eye.

microscopic–visible if using a microscope; not visible to the unaided eye.

occult blood–minute amount of blood which is detected by chemical tests or microscopic examination.

polyuria–excessive urination.

precipitation–test reaction during which a deposit falls to the bottom, thus separating out of solution.

pyuria–excessive white blood cells (pus) in the urine.

quantitative–test that determines how much of a substance is present as opposed to *qualitative*, which just indicates that a substance is present.

sediment–substance that separates out of solution and settles to the bottom.

specific gravity—weight of a substance as compared with an equal volume of water at the same temperature.

supernatant—the overlying liquid after a precipitate settles out of a solution.

void—urinate.

The urine is a very complex aqueous solution of several organic and inorganic substances, most of which are either waste products from body metabolism or products formed directly from the foods eaten. The most important organic materials are urea, uric acid, and creatinine. The chief inorganic ones are chlorides, phosphates, sulfates, and ammonia.

Certain materials appear in the urine only when disease conditions are present. These are proteins, carbohydrates, acetone, bile, and hemoglobin. Examinations should always be carried out on specimens that are fresh because the chemical composition is altered upon standing. Urine that cannot be examined immediately should be refrigerated.

Collecting the Specimen

In a hospital, the collection of routine urine specimens is the responsibility of the nursing service. They collect, label, provide the request form, and deliver the specimen to the laboratory. The laboratory personnel are then responsible for checking for complete labeling and agreement between the container and the request slip. Then they assign a laboratory number or log the specimen if required.

In a clinic laboratory, the patient is given a clean, dry container and asked to obtain a urine specimen. Directions must also be given for the location of the restroom and where the collected specimen is to be placed.

If a "clean catch" or midstream specimen is preferred, very clear instructions must be given to the patient so the specimen will be essentially free of contamination. The patient is instructed to cleanse the urethral area (if female) or glans (if male). The patient then begins to void into the toilet. This rinses the urethra. The midstream urine is collected into the clean container. The remaining urine is voided into the toilet. When the midstream specimen is to be used for bacteriological studies, a sterile container must be provided. When the specimen is received in the laboratory, it should be labeled properly, and a request form should be provided.

Figure 1 is an example of a correctly completed urinalysis request form. It is based on the following information:

10 URINALYSIS

PATIENTS NAME: Dan Reaves | AGE: 18 | CHARGE: $6.00 | PHYSICIAN ORDERING TEST: Gary Price | TO BE DONE: Routine urinalysis

CHART No.: 459-09 | ROOM No.: 303 | TIME DATE COLLECTED INITIALS: 9:00 a.m. 7/1/81 MM.

REQUESTED BY INITIAL: J.Y. | TIME: 8:30 AM | DATE: 6/30/81 | REPORT DATE & TECH: 7/1/81 M.N.

- [x] VOIDED
- [ ] CATH.
- [ ] URETER. [ ] L [ ] R
- [x] RT. URINALYSIS
- [ ] MICROSCOPIC ONLY
- [ ] OTHER

CENTRIFUGED: [x] YES [ ] NO

(2) COLOR: straw | (3) CHARACTER: clear | (4) PH: acid | (5) SP. GR. 1.0: 16

6 ALBUMIN: 10 mg%
7 SUGAR: neg.
8 ACETONE: neg.
9 OCCULT BLOOD:
10 BILE: neg.
11 PHENYLPYRUVIC ACID:

(12) WBC: 30-35 /HPF
(13) RBC: /HPF
(14) EP. CELLS: occ'l
(15) BACTERIA:
(16) CRYSTALS:
(17) MUCUS:
(18) HYALINE CASTS: 2-3 /LPF
(19) GRANULAR CASTS: /LPF
(20) OTHER CASTS: /LPF
(21) AMOR. URATES: /HPF
(22) AMOR. PHOSP.: /HPF
(23) YEAST: /HPF
(24) TRICH.: /HPF
(25) SPERM: /HPF
OTHER:

LABORATORY

Fig. 1. Example of a completed urinalysis form

1. The patient's name is Dan Reaves, 18 years old, room 303; chart # 459-09, Dr. Gary Price. Ward clerk Janie Yates filled out the request slip at 8:30 A.M. on 6/30/81 to be done in A.M. 7/1/81. Voided urine was collected at 9:00 A.M. by nurse aide Mary Moore and taken to the laboratory for routine urinalysis.

2. Mary Neal performed the urinalysis on 7/1/81 and did a microscopic examination on the centrifuged sediment with these findings: the urine was straw colored and clear. It had a specific gravity of 1.016 with an acid pH. The Labstix indicated 10 mg% albumin. Tests for sugar, acetone, and bile were negative. Microscopically there were 30-35 white blood cells/high power field, 2-3 hyaline casts/low power field, and an occasional epithelial cell. The fee was $6.00.

Urine specimens may be labeled to indicate the patient's name, the doctor ordering the test, the date collected, and whether routine or special tests are requested. Labels may also include the age of the patient and the chart number or hospital room number (see fig. 2).

There are several different types of urine specimen containers (see fig. 3). Most hospitals use plastic disposable containers. Reusable plastic, glass, or metal containers are commonly used in clinic laboratories.

Routinely, laboratories process urine specimens collected either as the first morning specimen or collected randomly. The *first morning* specimen is preferred because of the concentration. It is collected outside the laboratory by the patient or the nursing service. A *random* specimen is collected any time and has a variable dilution. If the patient collects the specimen at home, be certain that the laboratory provides the container. Patients must not be allowed to use dirty or contaminated receptacles.

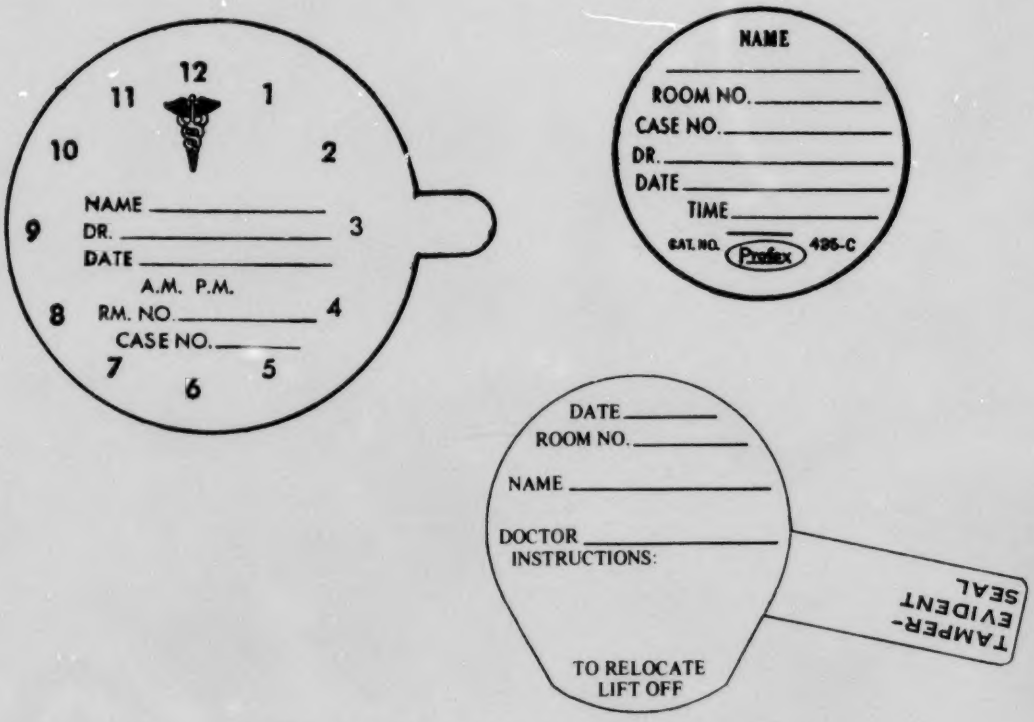


Fig. 2. Samples of labels for urine specimens

Performing the Urinalysis

Once the urine has been collected, properly labeled, and delivered, the laboratory is ready to begin the urinalysis. This is probably the most common laboratory procedure and consists of a series of tests performed on urine. A "routine urinalysis" is that series of tests selected by a particular institution to be performed on most urines. It generally includes both macroscopic and microscopic examinations, as well as chemical tests and measurement of the specific gravity.

Macroscopic examination is noted without the aid of a microscope and generally includes color and character. *Color* varies with concentration, urine pigments, and the presence of metabolic products. These common terms are used to describe the color of urine:

colorless—very diluted
straw—almost colorless
yellow—more concentrated; medications
orange—very concentrated; carotene, bile, or medications present
green or blue green—dyes or medications
pink red, or dark reddish orange—blood, hemoglobin, porphyrin, bacteria, vegetables, medication, or normal pigments after pH change
black or brown—bilirubin, iron, porphyrin, melanin, or medications

Character is the appearance or transparency of the urine and is reported as clear, partly cloudy (due to crystals, WBC, or blood), cloudy (many crystals, WBC, blood), or grossly bloody. The odor of the urine is not routinely reported unless it is particularly unusual.

The *specific gravity* is the weight (mass) of a measured volume of urine compared to the same volume of water at the same temperature. The specific gravity of water is 1.000. Normal ranges of urine specific gravity (sp. gr.) are 1.010 to 1.020. However, samples of urine taken at random may be far above or below these figures. Generally, the specific gravity is inversely proportional to the quantity of urine. In other words, a large quantity of urine would have a low specific gravity. Variations in specific gravity are of value in detecting whether the kidneys are functioning normally. It is low in cases of chronic nephrosclerosis, diabetes insipidus, and some functional nervous disorders. It is high in cases of fever and functional kidney disease, and is highest in diabetes mellitus. Dehydration and decreased fluid intake also yield an increased specific gravity.

Fig. 3. Urine specimen containers

The specific gravity is conveniently measured by using either a refractometer or a urinometer. The **refractometer** is a hand-held instrument that requires only a drop of urine (see fig. 4). It is especially useful when only a small quantity of urine is available for testing.

To use the refractometer, follow these steps:

1. Hold the instrument in a horizontal position.
2. Clean the surface of the cover plate and prism with lens tissue. Wipe lightly to avoid scratching the soft optical glass.
3. Place a large drop of the urine sample on the prism face, and close the cover plate.
4. Hold the instrument so that the prism window faces the light source.
5. Turn the eyepiece to provide a sharp focus of the scale.

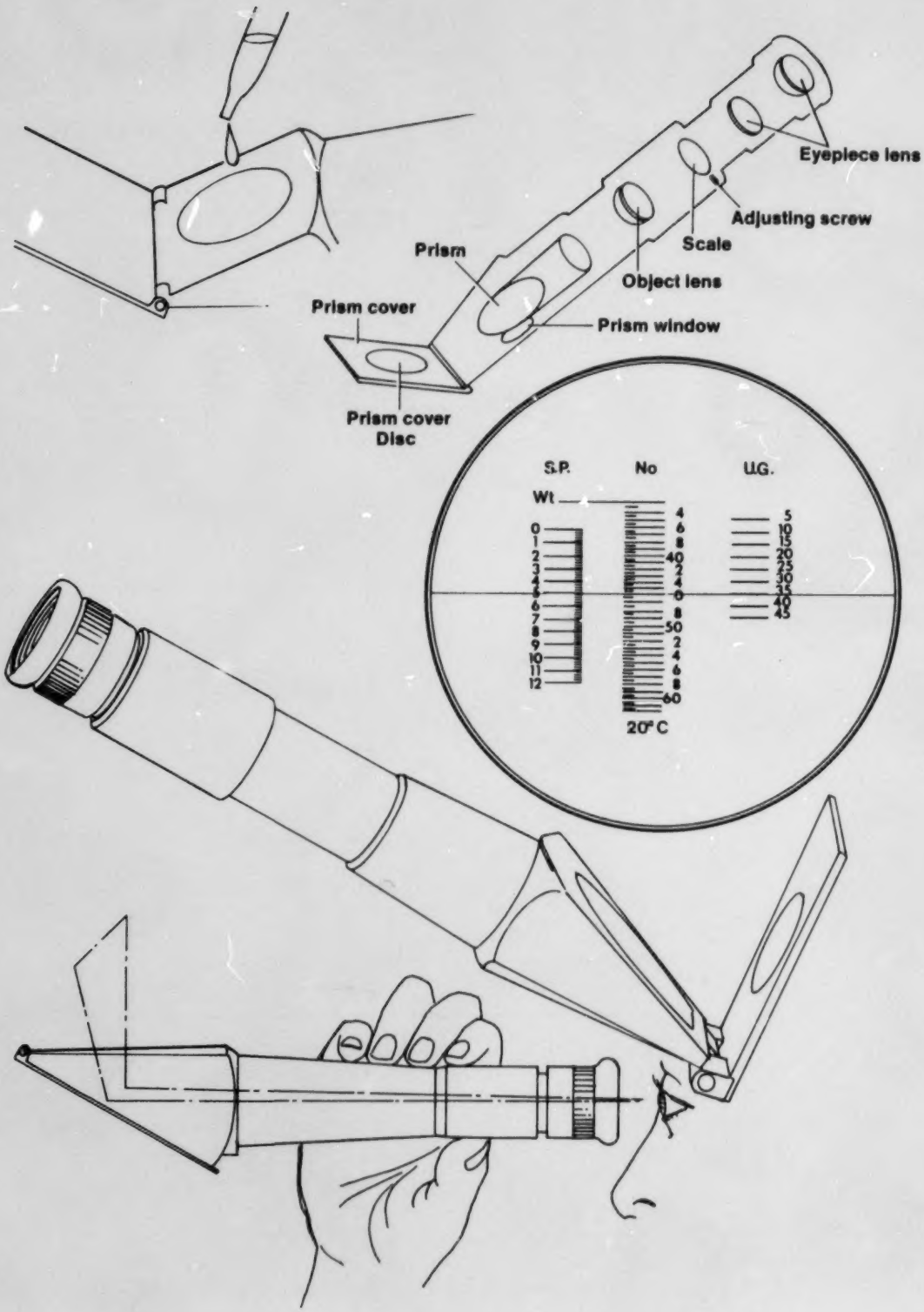


Fig. 4. Refractometer or hand protometer

The border line between the light and dark areas indicates the specific gravity of the urine. Always report the answer in four digits, such as 1.009 or 1.025.

When using the refractometer, always hold the instrument with the fingers close to the eyepiece, and hold the hinged prism cover against the surface with the thumb and forefinger. Do not *grasp* the prism section as this would raise the temperature and result in an incorrect reading.

When a **urinometer** is used to determine the specific gravity of urine, a larger amount of urine is required—usually about 15 ml. The urine should be mixed and then poured into the cylinder (see fig. 5). Place the urinometer (float) in the urine, and twirl it gently to be certain the float is free. It should not touch the side of the cylinder. It is also important that bubbles be removed from the surface of the urine. These are easily removed with a strip of filter paper. Read the bottom of the meniscus, and report the answer in four digits (e.g., 1.018). Make frequent checks with distilled water to determine the reliability of the urinometer or refractometer.

The *acidity* or *alkalinity* of the urine sample is referred to as the **pH.** Freshly voided urine is almost always acid, with a pH range of 4.8 to 7.0. Patients who eat high-protein diets may excrete more acid urine than those who consume mainly vegetables. A decreased pH is found in diabetic acidosis, fevers, pulmonary emphysema, diarrhea, dehydration, and during the use of some acidifying medications. Alkalinity (increase in pH) is found in chronic cystitis, certain genitourinary tract infections, pyloric obstruction, acute and chronic renal failure, and salicylate intoxication. Normally the urine becomes more acid with high-protein intake and sleep. It becomes more alkaline after collection and delay and after meals. The pH of the urine is easily determined using one of the commercial "stix" available. These provide a range of pH from 5 to 9 that includes a series of distinct color changes from orange to blue. The result is reported as acid, neutral, or alkaline.

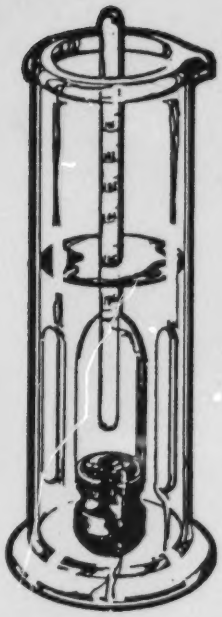


Fig. 5. Urinometer (float) inside cylinder

Chemical screening tests using "stix" are comprehensive, convenient, and economical. They give results that are reproducible. These are some of the more commonly used commercial multiple reagent strips that are manufactured and supplied by the Ames Division, Miles Laboratories, Inc. *Labstix* checks for pH, protein, glucose, ketone, and blood. *Bililabstix* checks for the same as Labstix plus bilirubin, *Multistix* checks for the same as Bililabstix plus urobilinogen, *N-Multistix* checks for the same as Multistix plus nitrites.

Also available commercially are single reagent tests: *Urobilistix* tests for urobilinogen, *Clinistix, Tes-tape, Diastix,* and *Clinitest* all check for glucose, *Ketostix* tests for ketones, *Ictotest* tests for the presence of bilirubin. All of these should be negative. Any protein, glucose, ketones, blood, bilirubin, urobilinogen, and nitrites present may indicate a pathological condition.

In addition, all children under the age of two should have their urine checked with a *Phenistix* (see fig. 6). If a color change ranging from

gray to blue-gray is detected, the presence of phenylketones (principally phenylpyruvic acid) is indicated.

To use any of the reagent strips, dip the "stix" in the fresh urine. Tap the strip against the container side. Observe the specified times, and read accordingly. Compare to the color chart. Record test results.

The urine specimen is now ready to be **examined microscopically.** Place 10 to 12 ml of well-mixed urine into a centrifuge tube, and centrifuge at 1,500 to 2,000 rpm for 5 minutes. Pour all of the urine out of the tube. Then place the tube in an upright position so that the fluid remaining on the sides flows to the bottom to dilute the sediment. Shake the sediment by tapping the tip of the tube on the hand. Place a small amount on a clean slide, and cover with a cover slip. (If too much is poured onto the slide, the elements will be distributed through many layers and may be missed on examination. If too little is poured onto the slide, the sediment may dry before the examination is completed.) Some laboratories prefer to use stained sediment. If this is the case at your training station, add one drop of crystal-violet safranin stain (Sternheimer Stain) to sediment, mix, and allow to stand for 3 minutes. Then place a drop on the slide, and cover with a cover slip.

Darken the field of the microscope by almost completely closing the diaphragm just beneath the stage. A well-darkened field is absolutely necessary if hyaline casts are to be found, because they are clear, colorless, and have a

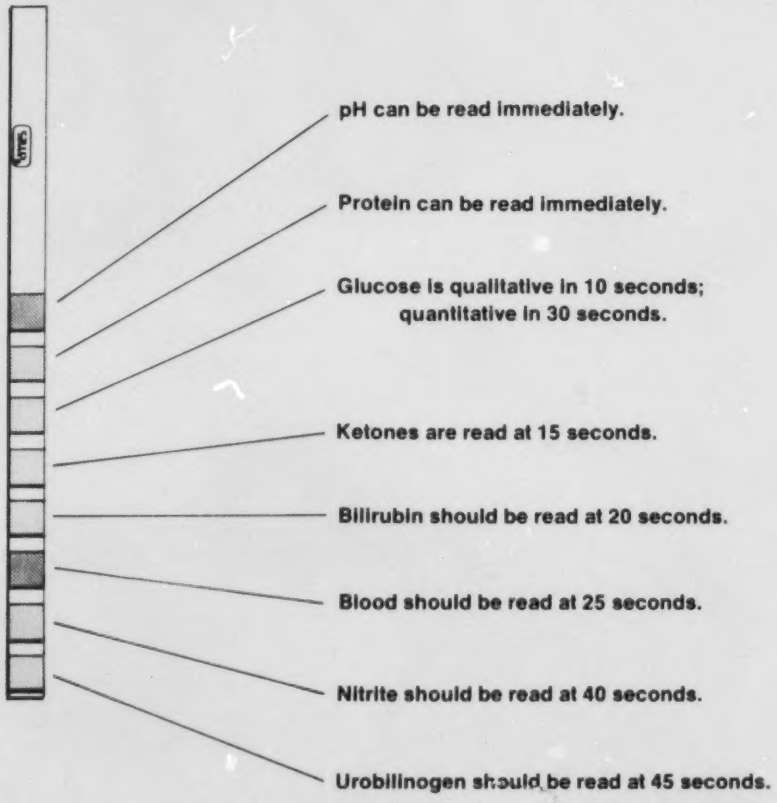

pH can be read immediately.
Protein can be read immediately.
Glucose is qualitative in 10 seconds; quantitative in 30 seconds.
Ketones are read at 15 seconds.
Bilirubin should be read at 20 seconds.
Blood should be read at 25 seconds.
Nitrite should be read at 40 seconds.
Urobilinogen should be read at 45 seconds.

Fig. 6. When a Phenistix is used, read at 30 seconds. (Courtesy Ames Division, Miles Laboratories, Inc.) (NOTE: The actual colors do not show in this illustration.)

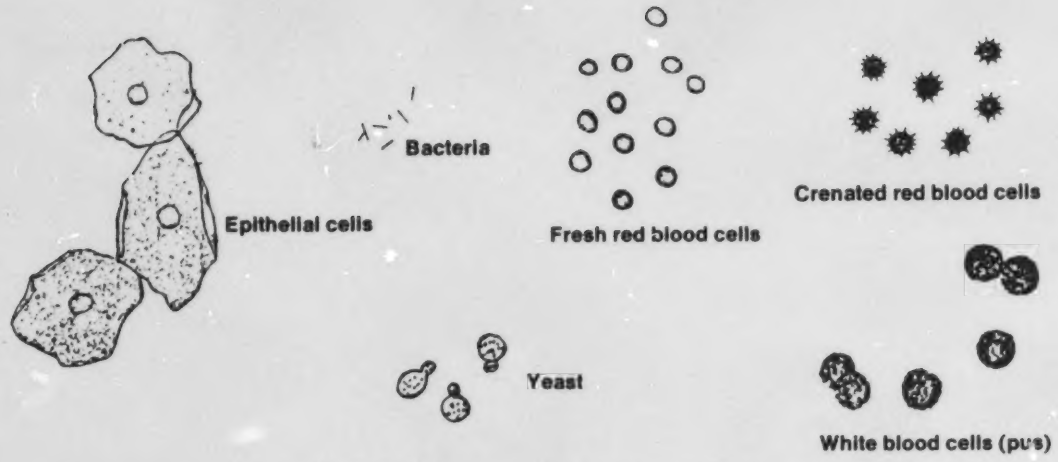


Fig. 7. Cellular structures found in urine sediment

low refractivity. First examine with the low power objective, then with the high power objective. Be careful to **avoid these errors:** careless transfer of sediment, use of too much light in the microscopic examination, use of high power only, drying of sediment, dirty equipment, scratches on the slide.

Examine using low power for casts, epithelial cells, crystals, amorphous material, and mucous threads. Use the high power to examine for erythrocytes, leukocytes, and bacteria. Report all microscopic findings according to the reporting guide for your particular institution. Some institutions report as few, moderate, many. Others report in average numbers (average should include ten fields) per low or high power field, such as 6/1pf or 15/hpf. Others may report in pluses, such as +, + +, + + +, + + + +.

Cellular structures found in urinary sediment (fig. 7) include the following:

1. *Squamous epithelial cells*—these are large, flat cells with small nuclei. They are derived from the urinary system, skin, or genitalia.

2. *Red blood cells (RBC)*—these indicate bleeding in the urinary tract or contamination from menstrual flow in females. They may also be present in fever, or through injury during catheterization.

3. *White blood cells (WBC)*—these may occur normally in females or children with 0-5/hpf in centrifuged specimens. Normal for males is 0-1/hpf. Increased numbers indicate infection.

4. *Bacteria*—these are not normally found in urine. They multiply rapidly when urine is left at room temperature. Their presence is significant if the collection is made using sterile technique.

5. *Spermatozoa*—their presence is not abnormal but should be reported.

6. *Trichomonas*—this is a parasite found in vaginal secretions. Their presence and frequency should be reported.

7. *Yeast*—these are singie-celled oval organisms, not normally present.

Several different kinds of **casts** may be found in urine sediment (fig. 8). The number is reported /lpf, but identification of type is confirmed on high power. Casts are cylindrical structures with blunt ends and parallel sides. They are composed of mucoprotein, and at times various other structures are included. Their presence indicates kidney disease. The type and frequency of cast is reported. These are some different kinds of casts found in urinary sediment: (1) *Hyaline* casts are cylindrical and transparent. (2) *Finely granular* casts contain fine granules. (3) *Coarsely granular*

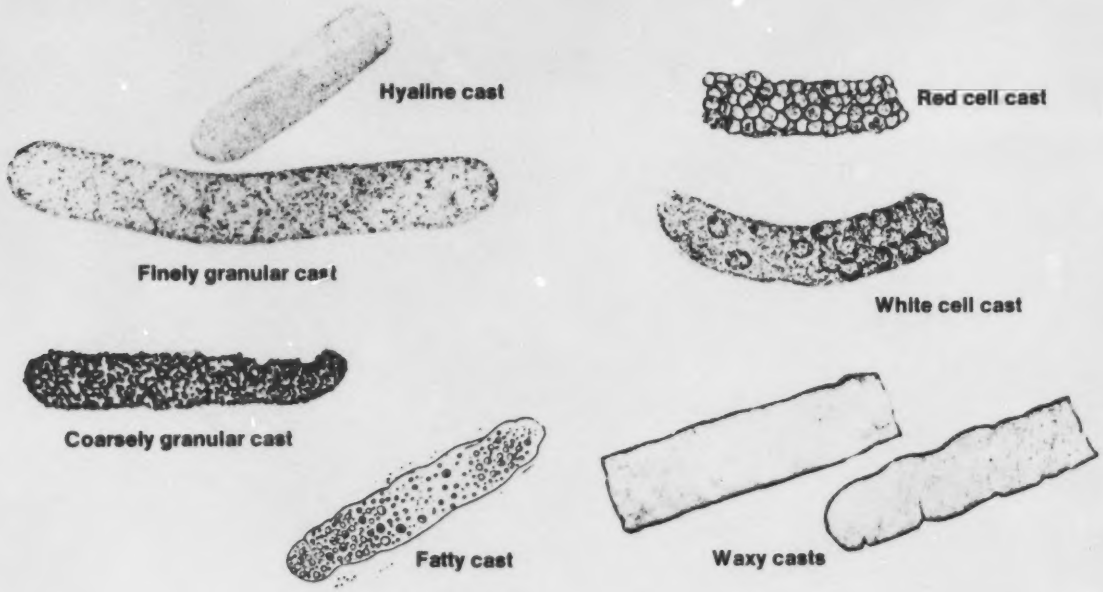


Fig. 8. Casts found in urine sediment

casts contain coarse granules. (4) *Red cell* casts are composed of RBC. (5) *White blood cell* casts (leukocyte casts) are composed mainly of leukocytes. (6) *Fatty* casts contain fat droplets. (7) *Waxy* casts are similar to hyaline casts, but are opaque and may be convoluted. (8) *Renal failure* casts are especially broad. They may be any of the previous types and may occur in "showers" (large numbers).

Cylindroids resemble hyaline casts, but have tapered ends and sides that are not parallel. They may be precursors of casts. *Mucous threads* are long, irregular, transparent structures that may have tapered ends (see fig. 9).

Amorphous and *crystalline* sediments generally have little significance. Most of them are substances normally present in solution that have precipitated either because of their excessive amounts or because of some alteration in the urine inside or outside the body. However, these crystals must be identified and their approximate number given.

These forms may be seen in *acid urine* (see fig. 10):

1. *Uric acid crystals*—shaped like plates, rhomboids, or clusters; may appear yellow or brownish.
2. *Amorphous urates*—may appear grossly as reddish sediment, and microscopically as fine granules that are often so numerous they obscure all other structures. They may disappear upon heating or the addition of caustic soda.
3. *Calcium oxalate cyrstals*—commonly appear as oval spheres, biconcave disks, dumbbells, or as a crystal with a cross inside.
4. *Tyrosine crystals*—appear as very fine needles, often arranged in sheaves.
5. *Leucine crystals*—appear as yellowish, oily looking spheroids.
6. *Cystine crystals*—appear as flat, colorless, hexagonal plates with unequal sides.
7. *Sulfanilamide derivatives*—may produce crystals that appear as prisms, rhombic plates, or needles in sheaves, but all of these forms tend to have rounded edges rather than extreme pointed edges. If

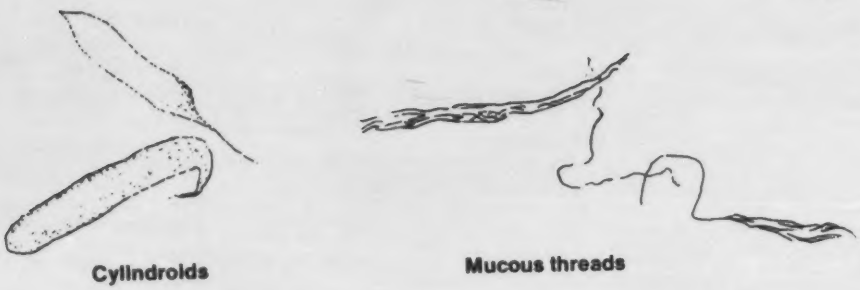


Fig. 9. Cylindroids and mucous threads

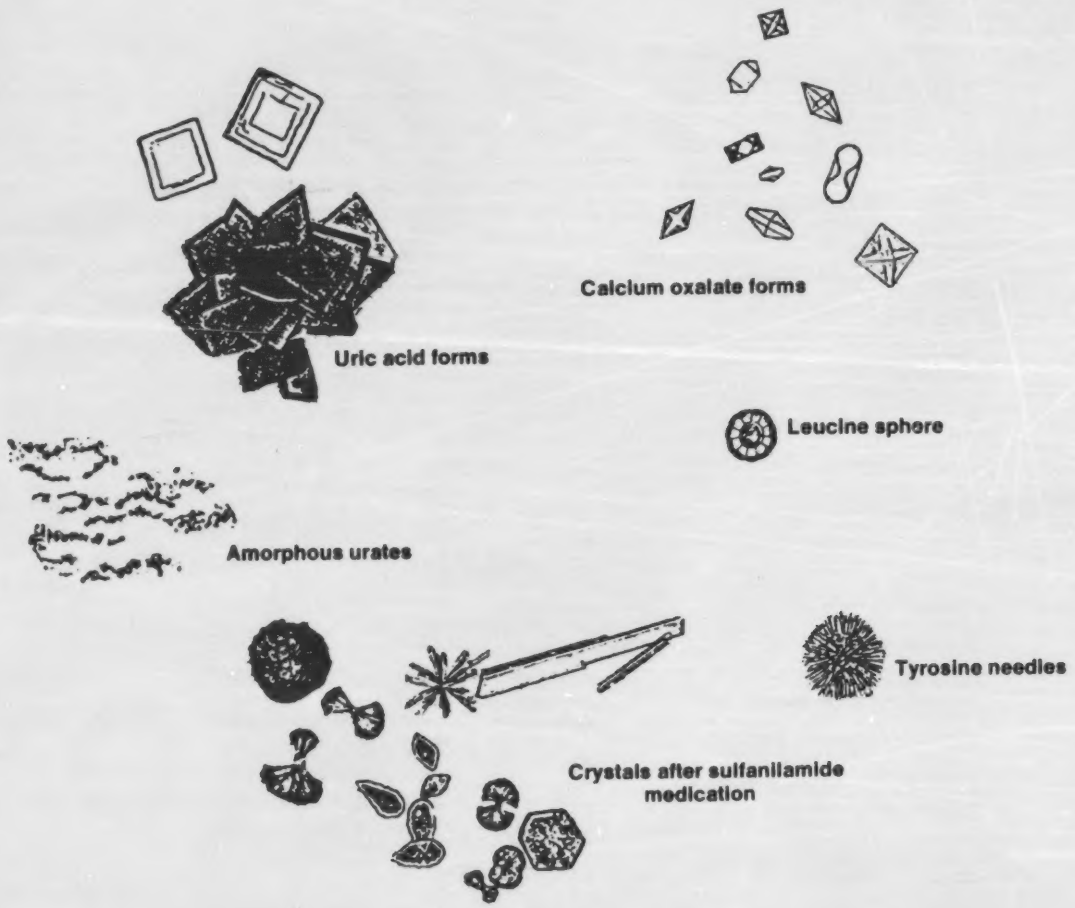


Fig. 10. Crystals in acid urine

blood is also present, this may be a clue that internal crystallization is taking place.

Common sediments that appear in *alkaline urine* include the following (see fig. 11):

1. *Triple phosphate or ammoniomagnesium phosphate crystals*—colorless and usually occur as some modification of a prism with three, four, or six sides.
2. *Amorphous phosphates*—appear as numerous colorless granules similar to amorphous urates found in acid urine. They are soluble in acetic acid.
3. *Calcium phosphate crystals*—appear as long prisms or needles or as large irregular plates.
4. *Calcium carbonate crystals*—may be hidden among amorphous phosphate granules or may be colorless spheres or dumbbells.
5. *Ammonium biurate crystals*—these are yellow spheres covered with spicules—"thorn-apple crystals."

Fat globules may occur in either acid or alkaline urine. They appear as highly refractive globules of various sizes. These stain black with osmic acid, and orange or red with Sudan III or scarlet red.

To the inexperienced eye, talc and starch granules may appear as crystals, and nylon, cotton, or vegetable fibers may be mistaken for casts or mucous threads.

Pregnancy tests are often performed on urine specimens. Years ago, laboratories kept rabbits and/or frogs. Reliable stock was difficult to maintain and did not always provide valid results. Much time and effort went into performing pregnancy tests when animals were used. In the early 1960s, chemical tesung that was simple, inexpensive, reliable, and yielded reproducible results became widely used.

Most pregnancy screening tests are qualitative, and many types are now commercially available. These are easy to perform and may even be purchased "over the counter" and run by the patient at home. Most of these tests measure the level of human chorionic gonadotropin (HCG) hormone excreted in the urine. The patient should be instructed to collect the first morning specimen in a clean container. (These conditions may cause inter-

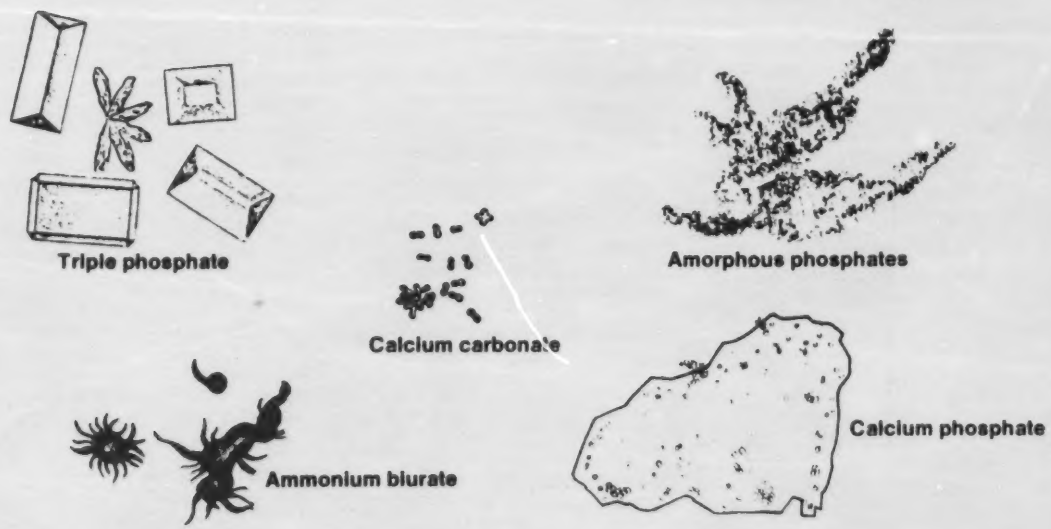


Fig. 11. Crystals in alkaline urine

ference in a pregnancy test: the presence of detergent residue in the collection container, medications taken by the patient, certain pathological conditions, menopause, or ectopic pregnancy.)

Some tests require filtered urine. If the sample is turbid, centrifugation may be required with testing performed on the supernatant. Some tests show agglutination (clumping) while others demonstrate precipitation in test tubes. Adequate controls must be prepared according to manufacturer's directions. The proper time period must be observed. A negative test result may occur in cases of very early pregnancy or if there is a low concentration of HCG. Negative tests should be repeated in 7 to 10 days as specified by the manufacturer. Quantitative tests may be performed on 24-hour urine specimens to determine any of these conditions: (1) threatened abortion, (2) hydatidiform mole, (3) ectopic pregnancy, or (4) fetal death.

UNIT 4

Hematology

Objectives: After completing this unit, you will be able to–

1. name the components of a CBC;
2. explain the procedure to perform an RBC and a WBC using a semiautomated instrument;
3. explain how to set up hemoglobin and hematocrit determinations;
4. calculate indices;
5. explain how to set up platelet counts manually;
6. set up an erythrocyte sedimentation rate, and calculate corrections when needed;
7. explain the sickle-cell disease test procedure;
8. explain how to perform a reticulocyte count;
9. prepare and stain blood smears;
10. describe stained leukocytes;
11. explain how to collect specimens for coagulation studies; and
12. name and explain bleeding and clotting times.

New Terms and Definitions

agranulocyte–those WBC which have few or no cytoplasmic granules.

capillary action–rise of fluid (blood) in narrow tubes.

CBC–complete blood count; that series of separate blood tests routinely done at a particular institution–usually includes a count of the various blood cells, measurement of hemoglobin, and calculation of indices.

ESR–erythrocyte sedimentation rate; rate at which red cells settle out in a specified time as a result of changes in the surface charge of red cells.

granulocyte–those WBC which have distinct granules in the cytoplasm.

HbS–abnormal form of hemoglobin which causes sickling of red cells, thus altering oxygen-carrying ability.

hematocrit–measurement of the percentage of the blood that is made up by the red blood cells.

hematology–study of blood; that department which concerns itself with blood cells and components.

hemocytometer–a small glass counting chamber with a microscopic ruled area for counting blood components; this counting chamber is placed on the microscope stage so cells can be counted accurately.

hemoglobin–chemical found in red cells which transports oxygen throughout the body.

heterozygous–condition in which genes are not identical; for example, HbS and HbA.

homozygous–condition in which genes are identical; for example, HbS and HbS.

interface–space or area that separates two materials that were previously mixed.

MCH–mean corpuscular hemoglobin; the average weight of hemoglobin in the RBC.

MCHC–mean corpuscular hemoglobin concentration; the weight of hemoglobin per volume of cells.

MCV–mean corpuscular volume; the average volume of the RBC.

platelet (thrombocyte)–a small piece of cytoplasm that has broken off from a larger mass; concerned with clotting mechanism in the body.

RBC–erythrocyte or red blood cell; contains hemoglobin.

reticulocyte–young red blood cell.

WBC–white blood cell or leukocyte; serves as a defense for the body by combating bacteria and viruses.

The Complete Blood Count (CBC)

Hematology means, literally, the study of blood. This is a reasonably good, though oversimplified, definition of the work that is performed in the Hematology Department. The most frequently ordered test in hematology is the Complete Blood Count, or "CBC" as it is better known. The CBC is routinely required on all patients admitted to a hospital. The CBC is actually a group of separate tests. Though the separate tests that make up a CBC may vary somewhat from one institution to another, a "typical" CBC at a hospital may consist of the following tests:

- White Blood Cell Count **(WBC)**
- Red Blood Cell Count **(RBC)**
- Hemoglobin **(Hgb)**
- Hematocrit **(Hct)**
- Red Blood Cell Indices:
  - Mean Corpuscular Volume **(MCV)**
  - Mean Corpuscular Hemoglobin **(MCH)**
  - Mean Corpuscular Hemoglobin Concentration **(MCHC)**
- Platelet Count **(Plt)**
- Differential White Cell Count **(Diff)**

Other tests frequently performed in hematology are the erythrocyte (red cell) sedimentation rate (known as **sed rate** or **ESR**), reticulocyte count (known as **retic count**), and the sickle-cell tests.

The CBC is a general indicator of a person's state of health. Yet a person may be ill even though all the tests that make up the CBC are within normal ranges. Generally, however, one or more of the tests will be abnormal (this may mean being too low as well as too high). Remember, when working with a living, biological system, the word *always* rarely applies. Following are some general statements relative to some of the tests that are included in the CBC.

White blood cells combat bacteria when an infection is present in the body. An abnormally high WBC usually means an infection. An extremely high WBC might indicate leukemia.

Red blood cells contain a chemical called *hemoglobin*, which is located inside the RBC and carries oxygen throughout the body. Persons who have too few red cells or too little hemoglobin inside the red cells are said to be "anemic." There are many possible causes of anemia. It may be as serious as leukemia or as simple as not getting enough iron in the food that is eaten. An RBC and a Hgb alone cannot diagnose the cause of anemia. Other tests must be performed to determine the actual cause of the anemia.

The *hematocrit* is a measurement of the percentage of the blood that is made up by the red blood cells. For example, a hematocrit value of 36% would mean that 36% of the total blood volume consists of RBCs. A "rule of thumb" relationship between the hematocrit and the hemoglobin is that, numerically, the hematocrit value is usually three (3) times the hemoglobin. For example, a hemoglobin of 11.0 grams should have a hematocrit value of 33%.

Thrombocytes or *platelets* are small pieces of cytoplasm that initiate (start) the clotting process when a person is injured. When the number of platelets in the blood stream is very low, internal bleeding may occur or the skin may develop severe bruises from only a slight

impact with an object. If a person with a very low platelet count does receive a cut or laceration, it may be very difficult to stop the resulting bleeding.

The differential white cell count and blood smear evaluation can provide much valuable information. A thin smear of the blood to be examined is made on a glass slide, stained, and then observed under the microscope. The various types of WBCs seen on the smear are categorized and tallied until 100 WBCs have been classified. Each cell type counted is then reported as a percentage. For example, if 23 of the 100 WBCs counted were classified as lymphocytes, then 23% lymphocytes would be reported. The size, shape, and appearance of the red blood cells are also observed, and written comments concerning any abnormalities are included in the report. The approximate number, size, and shape of the platelets are also observed and commented upon if abnormalities are seen.

Cell Count (RBC or WBC) Method

The procedure for performing cell counts, either RBC or WBC, will be discussed. *NOTE:* The Coulter methodology will be described for cell count technique. In the 1980 College of American Pathologists Proficiency Survey for Hematology, 2,815 of 3,724 laboratories participating nationwide used some type of Coulter instrument. That is about 76 percent. Thus, it is statistically more likely that the student will encounter a Coulter instrument at the training station. (No recommendation of nor preference for Coulter products is intended or implied.)

The blood cells suspended in an electrolytic solution can be sized and counted by passing them through an opening (aperture) through which an electric current is flowing. As the cells pass through the aperture and displace an equal volume of electrolyte, the electrical resistance in the path of the current changes. This results in corresponding current and voltage changes. The amount of this change is directly proportional to the size of the cell. As a specific volume of blood sample is drawn through the aperture by vacuum, each voltage change is counted. This count represents the number of cells in the sample.

To perform a WBC and RBC count using a semiautomated **Coulter** instrument, such as the Model ZF shown in figure 1, you need the following equipment:

- Isotonic buffered saline solution (diluent)
- Two snap-cap counting vials
- 20 µl pipette
- 10 µl pipette
- 0.1 ml pipette
- Lysing reagent

Pipette 10 ml of diluent into each of the two counting vials. Into one vial pipette 20 µl of the blood sample. Recap the vial, and mix by slowly inverting several times. The first vial contains a 1:500 dilution of the blood sample and will be used for the white cell count. The second vial contains a 1:50,000 dilution and will be used for the red cell count.

To the first vial (1:500 dilution) add three drops of lysing reagent to destroy the RBCs. Recap and invert to mix. To perform the actual counts on the Model ZF, the procedure is the same for both the red count and the white count (see fig. 1):

1. Lower the beaker platform **(A)**, and place the vial to be counted on the platform.
2. Slowly raise the platform, immersing the aperture tube **(B)**, and external electrode **(C)** into the sample.
3. Position the vial so that the aperture is clearly visible on the debris screen **(D)**.
4. Open the control stopcock **(E)** to a vertical position.
5. When electrical peaks appear on the sizing screen **(F)**, close the control stopcock **(E)** to the horizontal position. This initiates the counting process, and the numbers of the cells being counted will appear on the digital display **(G)**.
6. When counting stops, observe the final number on the digital display screen **(G)**.

There is a statistical probability that two cells may pass through the aperture at exactly the same time. To account for this, Coulter

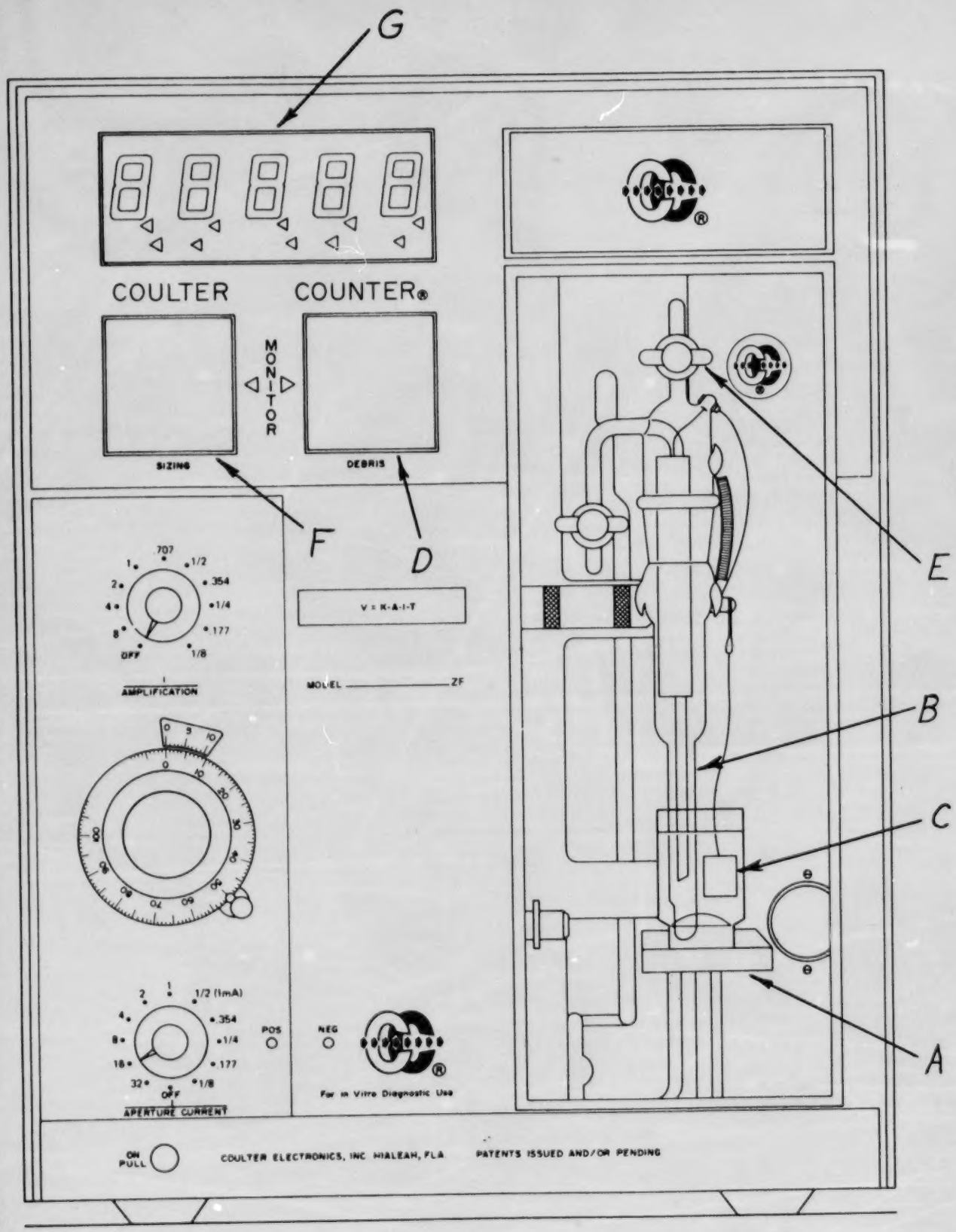


Fig. 1. Coulter Counter, Model ZF. (Reprinted by permission of Coulter Electronics, Inc.)

provides a Coincidence Correction Chart. To correct for coincidence, find the number obtained from the digital display screen on the chart, and read the corrected number in the adjacent column. *All* red counts are to be corrected. White counts *above* 10,000 should be corrected.

When a red count is performed, the digital display result will be in millions of cells per cubic millimeter of blood. For example, a reading of 4.32 means 4.32 million RBC per mm^3. Find 432 on the Coincidence Correction Chart, and read the corrected value (481). Thus, the value reported will be 4.81 million/mm^3.

White counts will be displayed in thousands. For example, if the display reads 6532, this means 6,532 WBC per cubic millimeter. For practical purposes, round off the answer to the nearest 100. Thus 6,532 becomes 6,500/mm^3. Remember to correct for coincidence on white counts above 10,000.

Sometimes a *manual cell count* is needed. However, the technologist will perform this test. The student probably will not have to develop this skill. Perhaps the supervisor at the training station will at some time demonstrate how to "charge" a counting chamber and will allow the student to practice RBC, WBC, and platelet counts.

Throughout this unit, you will see the symbol μ (mu) used with other symbols, such as μg, μl, or μg. This symbol μ stands for micro, which is a millionth part. Microgram is abbreviated μg; microliter, μl; and micromicrogram, $\mu\mu g$ (which is 10^{-12} grams).

To perform a hemoglobin determination using the **Coulter Hemoglobinometer,** you will need the following supplies and equipment:

- Counting vial
- Isotonic buffered saline (diluent)
- 10 ml pipette
- 20 μl pipette
- Hemoglobin lysing reagent

Pipette 10 ml of diluent into the vial. Pipette 20 μl of the blood to be tested into the vial. Add three drops of hemoglobin lysing reagent. Recap vial, and mix by inverting several times. Open the lid of the Hemoglobinometer, and pour the sample into the cuvette until it is completely filled. (A *cuvette* is a small glass container used to hold liquids that are to be examined photometrically.) Wait 5 seconds to allow any bubbles in the specimen to rise to the top of the cuvette. Close the lid. Read the results on the digital display in grams per deciliter (gm/dl).

The following supplies and equipment will be needed to perform a hemoglobin using the **Cyanmethemoglobin technique:**

- Spectrophotometer or colorimeter
- 5 ml pipette
- 20 μl pipette
- Test tubes (greater than 5 ml capacity)
- Optically matched cuvettes for spectrophotometer or colorimeter
- Drabkin's reagent
- Cyanmethemoglobin standard of known concentration

Pipette 5 ml of Drabkin's reagent into a test tube. Pipette 20 μl of blood to be tested into the tube with Drabkin's reagent. Mix by inverting, and allow to stand 5 minutes for color development. Set the spectrophotometer at a wavelength of 540 nanometers (nm). Into a matched cuvette, pipette 5 ml of Drabkin's reagent. Place cuvette in spectrophotometer, and adjust to 0.0 optical density (O.D.). Into another matched cuvette, pipette 5 ml of standard. Place in the instrument, and read the O.D. of the solution. Pour the prepared specimen, which has been standing for 5 minutes, into a matched cuvette, and place in the instrument. Read the O.D. of this "unknown" specimen. Calculate the results according to the following formula:

$$\frac{\text{O.D. "unknown"}}{\text{O.D. standard}} \times \text{concentration of standard} = \text{hemoglobin (gm/dl)}$$

To perform a hematocrit, the following equipment will be needed:

- Capillary tubes
- Sealing clay
- Microhematocrit centrifuge
- Microhematocrit reader

By capillary action, fill a capillary tube about three-fourths full of the blood to be tested. Seal the tube by pressing one end of the capillary tube into sealing clay. Place the sealed tube into one of the grooved slots in the head of the microhematocrit centrifuge. The sealed end of the tube should rest snugly against the rubber cushion at the outer edge of the centrifuge head. Screw on the cover plate securely. Close the centrifuge lid, and turn the timer on for 3 to 5 minutes (as determined by your training station). Remove the spun tube from the centrifuge, and read on any of several commercially available types of microhematocrit readers.

RBC Indices

The RBC indices are mathematical calculations of cell size and hemoglobin content. Mean corpuscular volume (MCV) is the average volume of the RBC. It is obtained by dividing the product of the hematocrit (Hct) times 10 by the RBC in millions:

$$\text{MCV in } \mu^3 = \frac{\text{Hct} \times 10}{\text{RBC (in millions)}}$$

For example, if the hematocrit is 45 and the RBC is 5,000,000/mm³, then:

$$\text{MCV} = \frac{450}{5} = 90\,\mu^3$$

Mean corpuscular hemoglobin (MCH) is the average weight of hemoglobin in the RBC. It is obtained by dividing the product of the hemoglobin (Hgb) times 10 by the RBC in millions:

$$\text{MCH in } \mu\mu\text{g} = \frac{\text{Hgb} \times 10}{\text{RBC (in millions)}}$$

For example, if the hemoglobin is 15 gm/dl and the RBC is 5,000,000/mm³, then:

$$\text{MCH} = \frac{150}{5} = 30\,\mu\mu\text{g}$$

Mean corpuscular hemoglobin concentration (MCHC) is the weight of hemoglobin per volume of cells and therefore is an indicator of the hemoglobin concentration of the average cell independent of cell size. It is calculated by dividing the hemoglobin (Hgb) by the hematocrit (Hct) and multiplying by 100:

$$\text{MCHC in \%} = \frac{\text{Hgb}}{\text{Hct}} \times 100$$

For example, if the hemoglobin is 15 gm and the hematocrit is 45%, then:

$$\text{MCHC} = \frac{15}{45} \times 100 = 33\%$$

The normal ranges for the RBC indices are these: MCV = 82–92; MCH = 27–31; MCHC = 32–40.

The RBC indices may be easily calculated using the **Best's Anemia Classifier,** as shown in figure 2.

To use the classifier, follow these steps:

1. Align the red central hairline of the transparent indicator with the red count on the outermost scale.

2. Firmly grasp the largest disk and the transparent guide together at the outer edge (periphery). Align the hematocrit and hemoglobin values with the red hairline of the

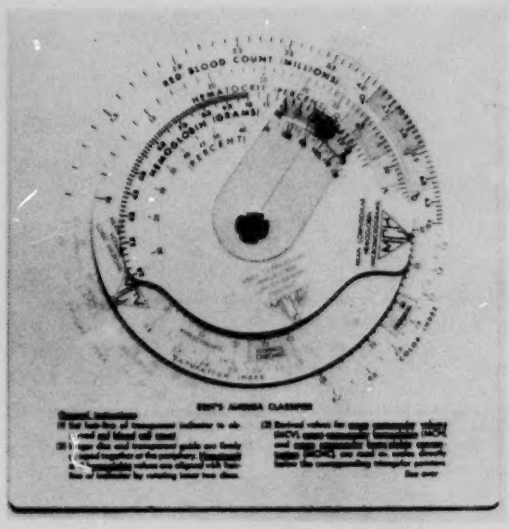


Fig. 2. Best's Anemia Classifier. (By permission of Clay Adams, Division of Becton, Dickinson and Co.)

indicator by rotating the two inner disks with the other hand.

3. Read the derived indices values on the scales directly below the corresponding pointers.

For example, given these values, you can easily determine the indices using the calculator: RBC = 5,000,000/mm³; Hgb = 16 gm; Hct = 48%.

MCV = 97 μ^3
MCH = 32 $\mu\mu$g
MCHC = 34%

Platelet Counts

Platelet counts may be performed using many different techniques and various new machines. However, the Unopette will be discussed here. You will need the following supplies and equipment:

- Unopette reservoir containing diluent for platelet determination
- Unopette capillary pipette–20 μl
- Microscope
- Counting chamber (fig. 3)
- Petri dish
- Counter

Pipette 20 μl of blood to be tested into the diluent reservoir according to the technique described by the manufacturer. Let stand 10 minutes to allow the red cells to hemolyze.

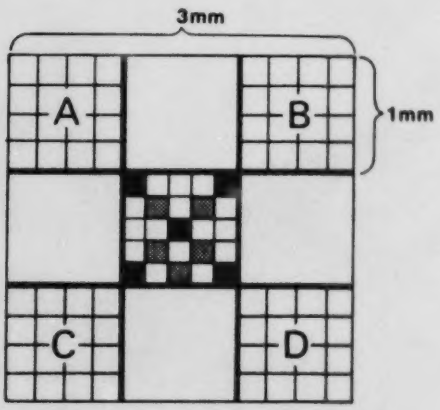


Fig. 3. Unopette counting chamber may be used for platelet counts.

Charge the counting chamber (refer to manufacturer's instruction). Place charged counting chamber into a petri dish, cover, and let stand 10 minutes to allow cells to settle. (The bottom of the petri dish should be covered with moistened filter paper to retard evaporation of the specimen.) Remove the counting chamber from the petri dish, and place it on the microscope stage. Locate the area to be counted on low power. Then switch to the high power objective, and count the platelets in all 25 small squares within the large center square. Multiply the number of platelets counted by 1,000 to get the total platelet count. If 250 platelets were counted, the total count would be:

250 × 1,000 = 250,000 platelets/mm³.

Sedimentation Rates (ESR)

Variations in the sedimentation rate result from changes in the surface charge of red cells. These changes are related to alterations in the plasma. Increased sed rates correlate, in a very general and nonspecific way, to inflammatory processes that may be occurring in the body. Sed rates (ESR) may best be used to monitor a patient's progress or therapy. One isolated ESR does not really tell the doctor a great deal.

To perform a Wintrobe method ESR, you will need:

- Wintrobe sedimentation tube
- Transfer pipette
- Wintrobe sed rate stand
- Timer
- Sed rate correction chart

Using a transfer pipette, fill a Wintrobe tube to the "0" mark with the blood to be tested. This is most easily done by placing the tip of the transfer pipette all the way to the bottom of the Wintrobe tube, then begin to allow the blood to flow into the tube out of the pipette. As the tube fills, raise the tip of the pipette so that it is barely submerged in the rising column of blood. The filled Wintrobe tube *must* be free of bubbles. Place the filled tube vertically in a Wintrobe sed rate stand. Set a timer for exactly 60 minutes. Read the level (in millimeters) to which the red cells have settled after 1 hour. This value is the "uncorrected" sed rate. To "correct" the sed

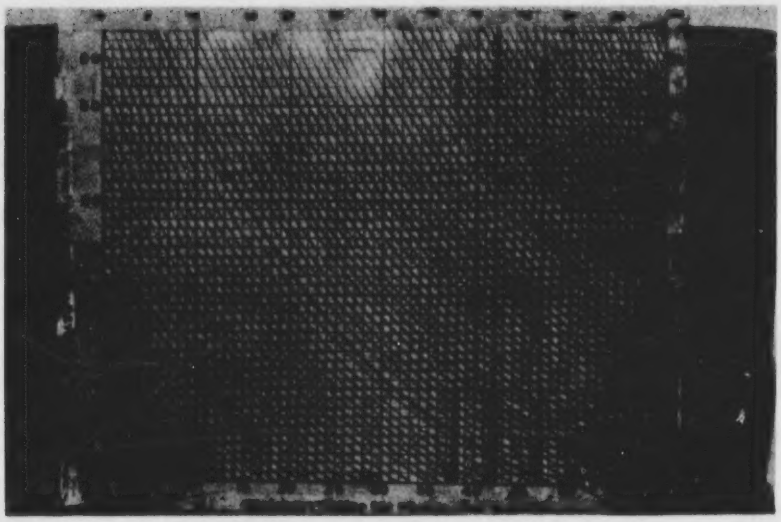


Fig. 4. Wintrobe sedimentation rate chart. (By permission of Lea and Febiger Publishers)

rate, the hematocrit value for the blood being tested must be known; so be sure to perform a hematocrit. Using the Wintrobe chart (fig. 4), first find the horizontal line corresponding to the "uncorrected" sed rate value. Then find the point where this line intersects (crosses) the vertical line corresponding to the hematocrit value. Select the curved line nearest to this point of intersection. Follow this curved line until it crosses the dark vertical line at 42 (if the patient is female) or at 47 (if the patient is male). The horizontal line corresponding to this last point is the "corrected" sed rate value.

Some training stations use a "wheel" such as the Clay Adams model shown in figure 5. The "uncorrected" ESR is located on the outer edge of the "wheel." Rotate the inner disk until the hematocrit occupies the approximate same space (either gray area or white area) across from the "uncorrected" ESR. Then read the arrow in the "corrected" area for either male or female. For example, an "uncorrected" reading of 15 and a hematocrit of 42 for a female would "correct" at 42. Actually, if the female has a hematocrit of 42% or the male has a hematocrit of 47%, that would be both the "uncorrected" and "corrected" ESR.

Newer methods of performing ESR determinations used in some laboratories require only 10 minutes. The method used is determined by the preference of the lab director and the budget of the lab.

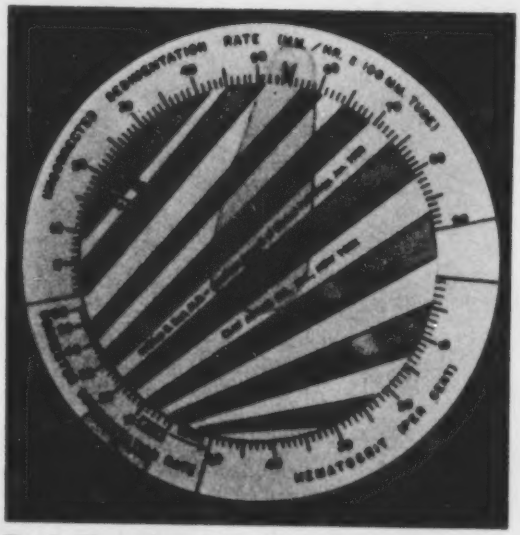


Fig. 5. Clay Adams wheel for finding sedimentation rate. (By permission of Clay Adams, Division of Becton, Dickinson and Co.)

Sickle-Cell Anemia

Sickle-cell disease is an inherited disease characterized by the presence of an abnormal hemoglobin (HbS). If a gene for HbS is inherited from each parent, the person will have *sickle-cell anemia.* This is a severe, disabling disease. If a gene for HbS is inherited from only one parent, the person will have the *sickle-cell trait.* This does not usually create problems unless the person is subject to reduced oxygen tension (such as during extreme physical exertion).

To check for sickle-cell anemia using the Sicklequik method, you will need the following:

- Two Sicklequik reaction tubes
- Two 0.1 ml pipettes
- Centrifuge
- Negative control (blood sample not containing HbS)

Label one reaction tube "patient" and the other reaction tube "control." Into the patient tube, pipette 0.1 ml of blood to be tested. Into the control tube, pipette 0.1 ml of the blood selected for your negative control. Recap the tubes, and shake them vigorously for 10 seconds. Allow the tubes to stand for 5 minutes, then centrifuge both tubes for 5 minutes at 3,400 rpm. (This test can be performed without a centrifuge. Allow the tubes to stand for at least 2 hours but not more than 72 hours before interpreting the results.)

If HbS is present, a dark red layer will form at the interface (point between upper organic phase and lower aqueous phase). The lower aqueous phase will be varying shades of straw, yellow, or amber or shades of red. If HbS is not present, the interface will be grayish and the lower aqueous portion will be dark red (see fig. 6).

Reticulocyte Count

Reticulocytes are young red blood cells. They are distinguished by blue granules inside the cell when stained with a supravital stain such as methylene blue. This test is used to determine whether appropriate numbers of new RBCs are being produced by the bone marrow. To perform a reticulocyte *(retic)* count, you will need the following supplies and equipment:

- Freshly filtered methylene blue N solution
- 12 × 75 mm test tube
- Dropper
- Glass slides
- Microscope

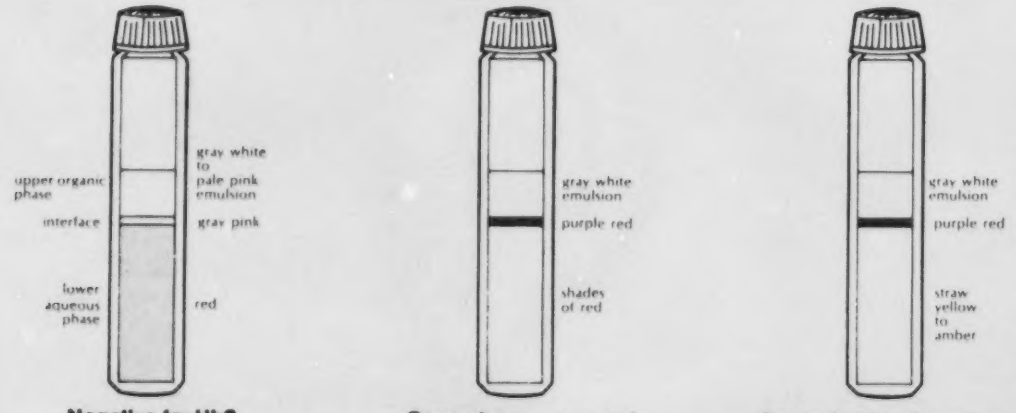


Fig. 6. Using the Sicklequik method to check for sickle-cell anemia

Using a dropper, place four drops of filtered methylene blue solution into a 12 × 75 mm test tube. Add four drops of the blood to be tested to the test tube containing the methylene blue solution. Mix by drawing the stain/blood mixture up into the dropper and expelling gently several times. Let the mixture stand for 10 minutes at room temperature. Mix again in the same manner, then prepare four thin smears on glass slides. Using an oil immersion lens, count 1,000 RBCs. Keep a separate tally of the retics seen while counting the 1,000 RBCs. (Count about 250 RBCs on each of the four slides.) Divide the number of retics seen per 1,000 RBCs by 10 to determine the percentage of retics. For example, 15 retics per 1,000 RBCs equals 1.5% retics.

Blood Smears and Differential Cell Count

The preparation of blood smears is very important to anyone working in the hematology department. Some laboratories prefer slide preparations. Others prefer cover slip preparations. We will be concerned with slides. The only equipment required is two glass slides and a drop of blood.

Place a small drop of blood on a glass slide, about ½ inch from one end (see fig. 7). Lay the slide on a flat surface. Place the end of a second glass slide, held at an angle of about 30 degrees, on the first slide in front of the drop of blood (fig. 7A). Pull the spreader slide back into the drop of blood. When the blood has spread about two-thirds the width of the slide, push the spreader slide forward with a steady, even motion (fig. 7B). The angle of the spreader and the speed of pushing may be changed to vary the thickness. Allow the smear to air dry. Ideally, the smear should have a "feather" edge. The technologist at your training station may show you some different ways to prepare smears.

Wright's stain or some variation of Wright's stain is used to stain the blood smear. Various laboratories will have their own staining technique. They may use automatic stainers, staining jars, or staining racks. We will describe a technique using trays. Place the air-dried blood smear slide on a staining tray with the blood film facing upward. Flood the smear with Wright's stain solution. Let stand for 1 to 2 minutes. Then add an approximately equal volume of buffer solution to the slide. Blow the surface of the stain-buffer mixture to ensure proper mixing. Let stand for 3 to 5 minutes. Flood with tap water to remove the stain-buffer mixture. Wipe off the underside of the slide. Stand the slide on end in a drying rack to drain and air dry.

The hematology department can give the physician much valuable information by reporting completely and accurately what is observed while doing the **differential cell count (Diff).**

Leukocytes are those blood cells which take up the purple-blue-pink-red stain. Leukocytes (WBC) are of two general varieties—*granular* (those which have distinct granules in the cytoplasm) and *agranular* (those which have few or no cytoplasmic granules).

The granular leukocytes *(granulocytes)* can be distinguished from each other by the color of their granules. *Eosinophils* (eos) have distinct red cytoplasmic granules (see fig. 8). *Basophils* (baso) have large blue cytoplasmic granules. *Neutrophils* have a light pink cytoplasm with

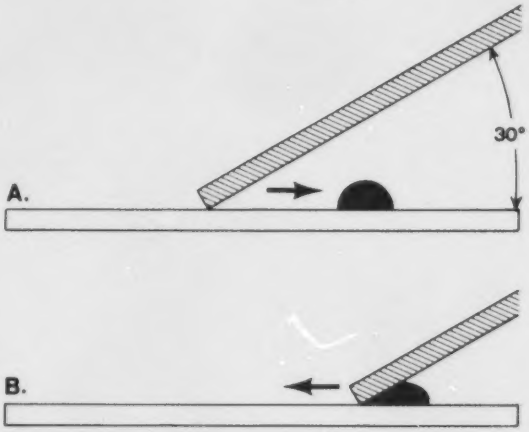


Fig. 7. Preparation of blood smears using slides

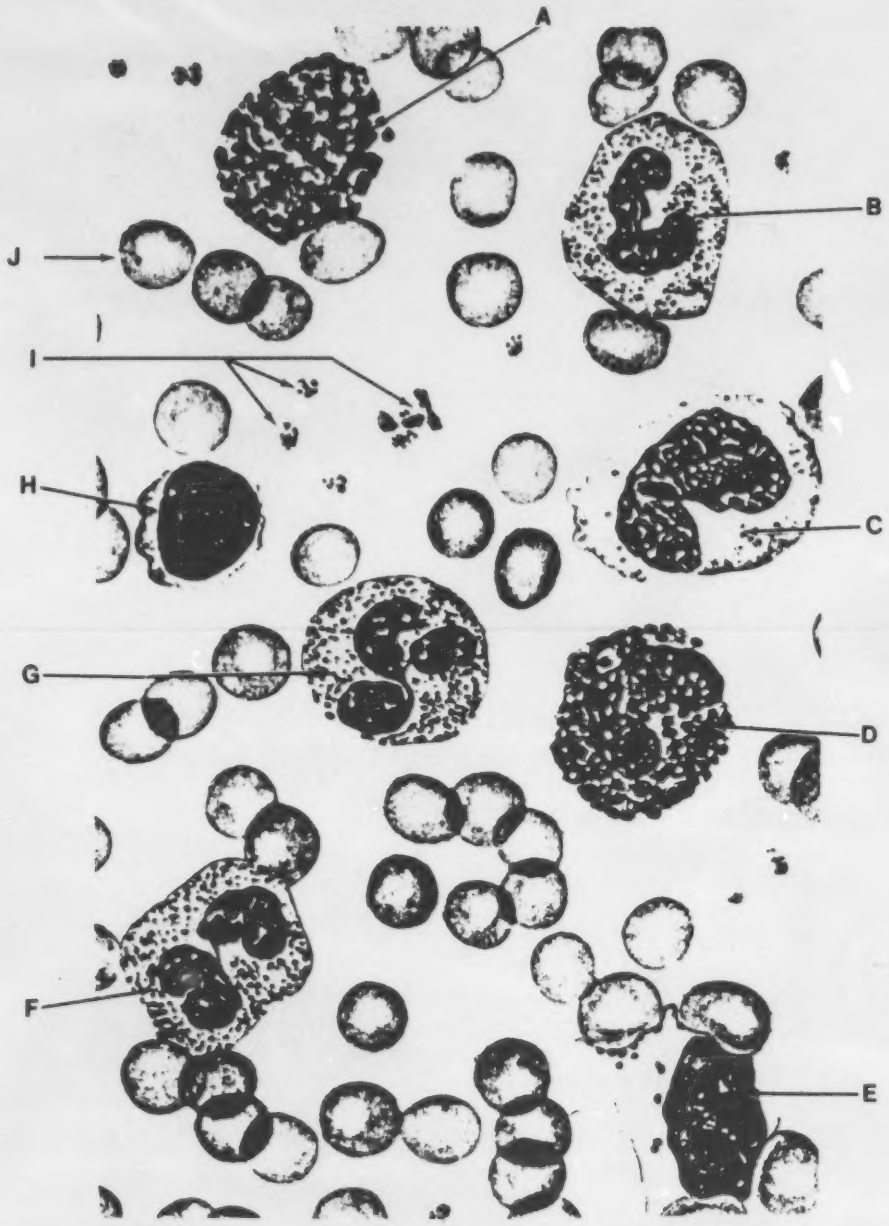


*Fig. 8. Basophil (**A**), band (**B**), monocyte (**C**), eosinophil (**D**), lymphocytes (**E** and **H**), neutrophils or segs or polys (**F** and **G**), platelets (**I**), and RBC (**J**)*

small, numerous, evenly distributed light pink to bluish black granules. As the granular leukocytes mature, the nucleus tends to become separated into distinct lobes (which take up the dark stain) with a narrow filament connecting the lobes. These are called *polys* or *segs*. A *band* is a neutrophil whose nucleus has lengthened but has not separated. A *juvenile* has a slightly indented nucleus.

The agranular leukocytes are the lymphocytes and monocytes. The *lymphocytes* (lymphs) have a blue cytoplasm, nonsegmented nuclei. They may or may not have granules. The *monocytes* (monos) have variable shapes and a dull gray blue cytoplasm with numerous, fine, lightly stained, evenly distributed granules. The nucleus has brainlike convolutions and may be linear, E-shaped, or horseshoe-shaped.

Place a drop of immersion oil on the stained slide, and observe on high dry for anything unusual. Switch the objective to the oil-immersion lens, and count 100 WBCs. After 100 cells have been counted, a noise will sound (depending on which kind of WBC Differential Counter is used). Be certain to *clear* the readout before performing another Diff. The technologist reports not only the percentage of each kind of WBC, but also such terms as macrocytic, microcytic (larger/smaller than normal), poikilocytosis (various shapes), anisocytosis (all sizes on the smear), and polychromatophilic (variation in color) to describe the RBCs. Figure 9 shows various RBCs that might be seen on a smear.

Coagulation Studies

The collection and handling of specimens for coagulation *(coag)* tests is very important. By means of a patented system, blood is aspirated from the vein directly into evacuated glass tubes containing specified amounts of anticoagulant. These precalibrated tubes draw a specified amount of blood to yield the proper 9:1 ratio of blood to anticoagulant. For plasma recalcification time, prothrombin time, activated partial thromboplastin time (PRT, PT, APTT), fibrinogen assay, protamine sulfate, euglobulin lysis time, factor identification, factor assay, and any additional tests on plasma, 4.5 ml whole blood is added to 0.5 ml of 3.8% sodium citrate.

After proper identification of the patient, a clean (quick and accurate) venipuncture is necessary with minimal tourniquet pressure preferred. Blue-top (citrate) tubes for the coag test should be collected after first drawing the EDTA (lavender-top) tubes for cell counts and the red-top tubes for chemistry and serology.

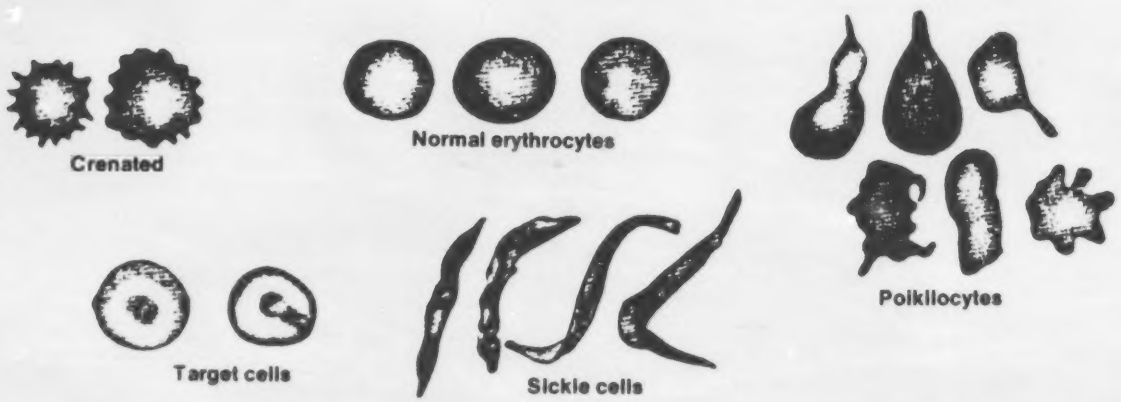


Fig. 9. Various RBCs that might be seen on a smear

(The top colors refer to Becton-Dickinson Vacutainers.)

The first tube is most likely to be contaminated with small amounts of tissue thromboplastin, which can activate the extrinsic coagulation pathway and lead to variable coag results. Prompt and proper mixing of all blue-top tubes must be done to bind all the calcium immediately.

The volume of blood aspirated must be correct for tube size and amount of anticoagulant. Comparison tubes, calibrated and marked to show proper filling, should be available near the centrifuge. Tubes must also be checked for clots and hemolysis.

The tests to be performed will determine the time and speed of centrifugation. If *platelet-rich* plasma is desired (for PRTs), spin the capped tubes at 1,500 rpm for 5 minutes. When *platelet-poor* plasma is desired (PTs, APTTs, fibrinogen assays, etc.), spin the capped tubes at full speed (2,000 + rpm) for 10 minutes. When *multiple tests* other than PRTs are to be performed and platelet-poor plasma is desired, a disposable serum-plasma separator device, such as Sure-Sep, may be inserted before centrifugation. Precaution should be exercised to avoid skin contact with the blood when inserting a separator device.

Since coagulation tests are enzymatic procedures, reaction temperature must be controlled at 37°C by instrumentation. Other variables in coagulation studies are the choice of anticoagulant (citrate is preferred rather than oxalate) and loss of CO_2 and rising pH (due to unstoppered vs. stoppered tubes). If the specimen cannot be immediately tested, parafilm-sealed tubes may be stored in the refrigerator for 1 to 4 hours. If testing will be delayed longer than 4 hours, plasma should be placed into stoppered tubes and frozen.

The *bleeding time* is that time between the making of a small incision and the moment bleeding stops. It is a test of platelet function and is frequently used as a presurgical screening test. Many methods have been developed for performing this test. Among them are the Ivy, the template, and the Duke, or modifications of these. The original bleeding time that Duke described was performed by making puncture wounds on the forearm while a constant pressure of 40 mm was held on that arm by using a sphygmomanometer. The modified Duke method uses the finger, and depends on the elasticity of the skin and capillary vessels, the efficiency of tissue fluids, and the chemical and mechanical action of the platelets.

Methods for performing clotting times also vary from laboratory to laboratory. Perhaps the most accurate method is the Lee-White Clotting Time. However, the Dale and Laidlaws Capillary Clotting Time is widely used. The Lee-White method uses venous blood. The capillary method uses blood from the finger, toe, earlobe, and so on. It is important to use a rapid, free flow of blood that has not been obtained by squeezing or manipulation of the puncture wound.

Coagulation consists of changing fibrinogen (one of the plasma proteins) into fibrin by the use of thrombin and calcium salts. Fibrinogen, calcium salts, prothrombin, and antiprothrombin are normal, constant constituents of the plasma. Prothrombin and antiprothrombin are held in inactive combination, so coagulation within the vessels does not occur. Thromboplastin which accelerates coagulation is not present in the circulating blood but is located in the tissue fluid. When blood escapes from vessels, it comes in contact with the thromboplastin in the tissues. This neutralizes the antiprothrombin, which sets the prothrombin free to react with the calcium salts. The prothrombin is transformed into thrombin. This, in turn, transforms fibrinogen into fibrin, which then forms a meshwork of threads to entangle the blood cells. It is thought that disintegrating platelets contain enough thromboplastin to liberate the antiprothrombin and start the coagulation process.

thromboplastin + calcium salts + prothrombin → thrombin

thrombin + fibrinogen → fibrin

Blood for prothrombin time and partial

thromboplastin time must be collected in a test tube containing a special anticoagulant. Most laboratories use a 9:1 ratio of blood to 3.8% sodium citrate. Other anticoagulants used are sodium oxalate and EDTA (ethylene-diamine tetra acetic acid). A 3.8% solution of sodium citrate may be obtained prepackaged within Vacutainers in sealed cans (from Becton-Dickinson Company, Rutherford, New York). They are stored at room temperature and are stable for a year.

The *prothrombin time* is also referred to as P.T. protime, prothrombin time test, or a P.T. test. A prothrombin time is a measure of the clotting ability of a series of combined coagulation factors in plasma. Prothrombin is a protein substance produced by the liver and required for the clotting of blood. Although the prothrombin time does not directly measure the amount of prothrombin, the test is a suitable clinical means of determining the presence and functioning ability of prothrombin.

Patient plasma is added to a mixture of calcium and thromboplastin to form a clot. The time interval between the addition of the plasma to the calcium-thromboplastin mixture and the appearance of the clot is called the prothrombin time. Blood is collected in a 9:1 ratio of blood to sodium citrate. These are thoroughly mixed so that the calcium in the blood binds with the anticoagulant and is thus removed from the plasma. Since calcium is one of the factors necessary in coagulation, it must be restored to the system during the test procedure.

The function of the thromboplastin in the protime test is to assist the prothrombin in converting to thrombin so a clot can form. Normal values for a protime are 10 to 16 seconds. The protime is often used in the diagnosis of disease. It is more useful during the treatment of blood clots. When a patient has a blood clot, he/she receives a series of treatments with an anticoagulant, such as dicumarol or heparin. These drugs delay coagulation—preventing existing blood clots from expanding and new clots from forming. Since the anticoagulant delays clotting, it causes the protime results to be above normal. This necessitates the monitoring of the patient's clotting time (therapeutic range is 28 to 40 seconds). If the protime rises above 40 seconds, the patient could hemorrhage. If the protime falls below 28 seconds, the treatment may be ineffective, causing old clots to expand or new clots to form. The protime may be performed by the following methods. The *manual* method is the tilt tube method or wire loop method. Automated methods are Fibro System, Electra 750, Hemochron, or Data-fi. No matter which method is used, the protime is a valuable test and must be performed with extreme care.

The activated partial thromboplastin time (APTT) is the single most useful procedure available for routine screening of coagulation disorders. The APTT measures the coagulation factors present. Calcium in whole blood is bound by the anticoagulant as in the protime. The plasma, after centrifugation, contains all the intrinsic coagulation factors (except calcium and platelets). Calcium, partial thromboplastin (a platelet substitute), and kaolin are added to the plasma. The time required for the plasma to clot is the APTT. Normal range for an APTT is 22.5 to 32.5 seconds (by the Electra 750 method). When the APTT is longer than normal, this may be due to a deficiency in one of the coagulation factors, or it may be due to anticoagulants circulating in the patient's plasma.

UNIT 5

Serology

Objectives: After completing this unit, you will be able to–

1. name and perform tests for syphilis, such as VDRL and RPR;
2. perform and/or identify other serological tests to detect–
   a. C-reactive protein
   b. rheumatoid arthritis
   c. infectious mononucleosis
   d. lupus erythematosus
   e. febrile agglutinations
   f. ASO titers
   g. cold agglutinins
   h. Coombs (antiglobulin) antibodies; and
3. assist with calibration of tabletop centrifuges.

New Terms and Definitions

agglutination–the collection into clumps of the cells that are distributed in a fluid.

agglutinin–specific antigen used in agglutination tests.

antibody–proteins developed within the body in response to a foreign substance (antigen), often providing immunity to disease.

antigen–substance that induces the formation of antibodies.

atypical–irregular; not conforming to type.

buffy coat–light-colored layer of a blood clot between the plasma and RBC; contains mostly WBC.

febrile–pertaining to a fever.

flocculation–colloid phenomenon in which the disperse phase separates in discrete, usually visible particles rather than in a continuous mass.

fluorescent–luminous when exposed to other rays.

F.U.O.–abbreviation for fever of undetermined origin.

hemolysis–dissolution of red blood cells with the liberation of hemoglobin; destruction of red cells by a lysin.

heterophil antibody test–test for infectious mononucleosis using a series of diluted patient sera and washed sheep red blood cells.

inactivation–to render inert by use of heat; to destroy biological activities by heat.

in vitro–that which can be observed or demonstrated in a test tube as opposed to *in vivo,* which occurs within the living organism.

lysis–destruction of cells by a specific lysin.

rickettsia–minute rod-shaped or coccobacillary microorganisms that are parasites and cause disease in vertebrates; intermediate between bacteria and viruses.

serology–study of antigen-antibody reactions *in vitro.*

serum sickness–condition which develops following administration of antisera or drug therapy.

spirochete–slender, spiral, motile microorganism.

stirrer–device used to mix serum/cells with reagents such as specific antigens that are added to check for presence of antibody; may be toothpick, applicator stick, glass stirring rod, and so on.

titer–highest dilution of serum causing agglutination or strength of serum.

Serodiagnosis

Serology is the study of antigen-antibody reactions *in vitro* (can be observed in a test tube or on a glass slide). When a patient has an infectious disease, certain substances **(antibodies)** are formed in the tissues that have the ability to destroy or affect the disease-producing agent **(antigen).** These substances are found in the blood plasma and lymph and appear in the serum after the blood coagulates. Generally, these "antibodies" or "immune bodies" are *specific*, and the demonstration of them indicates the presence of an infectious disease caused by a specific antigen. The antibody is often easier to detect than the disease-producing agent itself. The antibodies may appear immediately after the onset of the disease, or detection may be delayed until the disease is advanced. The antibodies remain for a variable time after recovery from the infectious disease.

Nonspecific antibodies are antibodies that are formed in response to an agent other than the bacterium that causes a given disease. For example, antibodies developed in response to tissue damage in syphilis are nonspecific.

The presence of specific antibodies may be detected by tests that demonstrate one of the following results: agglutination, flocculation, bacteriolysis, precipitation, complement-fixation, hemolysis, or the presence of opsonins.

Initially, a *serology* test indicated a test for syphilis. Now the area has been expanded to include tests for other pathological conditions characterized by a rise in antibodies.

Syphilis

Syphilis is caused by the spirochete *Treponema pallidum.* In response to this disease process, two antibodies develop. The treponemal antibodies are specific and are developed against the spirochete. The nonspecific antibodies are developed in response to tissue damage by spirochetes and are called *nontreponemal antibodies.*

The three most commonly used tests for syphilis are the (1) FTA-ABS (Fluorescent Treponemal Antibody-Absorption Test), (2) the VDRL (Venereal Disease Research Laboratory), and (3) the RPR (Rapid Plasma Reagin). The FTA-ABS test is specific and is performed to confirm reactive sera. This test is performed in large laboratories, reference laboratories, and state facilities. The VDRL test is nonspecific. It requires inactivated serum and can be done easily in most laboratories. The RPR test is also nonspecific. It can be performed on plasma and is more sensitive but less specific than the VDRL. The RPR does not require inactivation of serum and can be done easily and rapidly in any laboratory. Less frequently used tests for syphilis are the Wasserman, Kahn, Colloidal Gold, TPI (Treponema Pallidum Immobilization), Plasmacrit, and Dark Field Microscopy.

Procedure for VDRL Test

You will need the following materials before you begin the test:

- 56°C water bath or heating block
- Centrifuge
- Test tubes
- 0.5 ml pipettes
- 1 ml pipette
- Ceramic ringed slides
- Syringe
- 18-gauge needle without bevel (calibrated to deliver 60 drops/cc antigen)
- 2 ml pipette
- 5 ml pipette
- 30 ml round-bottom, glass-stoppered bottle

- VDRL antigen
- Mechanical rotator
- Microscope
- Control sera
- VDRL buffered saline

To **prepare the serum,** follow these steps:

1. Collect blood, and centrifuge to separate serum.
2. Decant serum, and inactivate by placing tubes of sera in water bath or heat block for 30 minutes at 56°C.

Prepare VDRL antigen while the serum inactivates.

To **prepare the VDRL antigen,** follow these steps:

1. Pipette 0.4 ml buffered saline onto the bottom of a 30 ml round-bottom glass-stoppered bottle.
2. Add 0.5 ml VDRL antigen drop by drop (taking 6 seconds) from the lower half of a 1 ml pipette. Hold the pipette in the upper third of the bottle. Be careful to add the antigen directly onto the saline while continuously and gently rotating the bottle on a flat surface. The center of the bottle should make a 2-inch diameter circle three times per second.
3. Blow the last drop of antigen from the pipette without touching the pipette to the saline.
4. Continue to rotate the bottle for 10 seconds.
5. Add 4.1 ml of the buffered saline from a 5 ml pipette. Place the glass stopper on the bottle, and shake it from bottom to top for 10 seconds. The antigen is stable for use only on the day prepared.

To **perform the VDRL test,** follow this procedure:

1. Pipette 0.05 ml inactivated serum into one of the rings of the slide.
2. Place controls that have also been inactivated into the marked areas of the slide.
3. Add 1 drop of antigen suspension with an 18-gauge needle held vertically (so it will deliver 1/60 cc per drop).
4. Rotate slide on a mechanical rotator (fig. 1) for 4 minutes at 180 rpm.
5. Read microscopically on low power (10X) immediately.
6. Report as reactive (large clumps that are visible to the naked eye), weakly reactive (small clumps), or nonreactive (no clumping).

Procedure for RPR Test

To perform the RPR test, follow these steps:

1. Place a drop of plasma or serum onto the circle on the RPR card using a capillary tube and rubber bulb, and stirrer (fig. 2A).
2. Spread the drop throughout the circle with the stirrer (fig. 2B).
3. Treat the control sera the same as the patient serum.
4. Add 1 drop of well-shaken antigen from antigen dispensing bottle (fig. 2C).
5. Place on the mechanical rotator, and cover with humidifying cover.
6. Rotate 8 minutes.
7. Read macroscopically, and report as reactive or nonreactive (fig. 2D).

C-Reactive Protein Test

C-Reactive protein is an abnormal protein found in persons with infectious and inflammatory conditions. The CRP test is nonspecific.

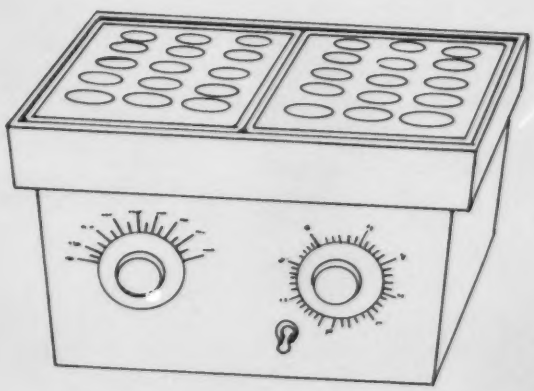


Fig. 1. Mechanical rotator used in VDRL test

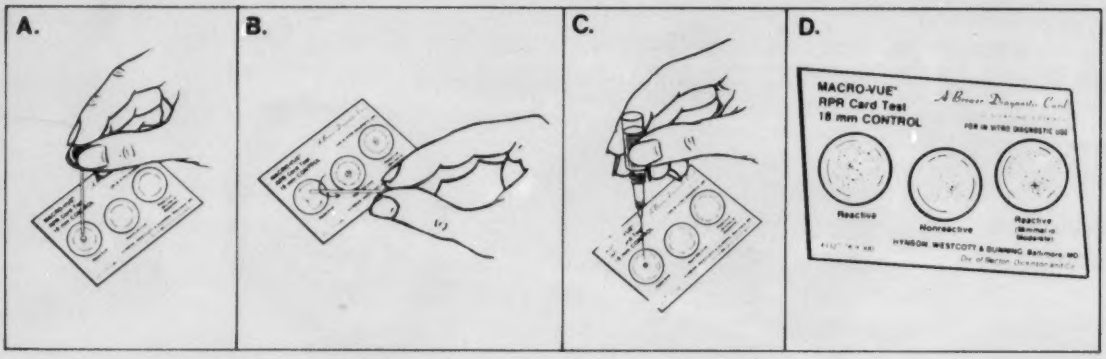


Fig. 2. Procedure for RPR test

The abnormal protein appears within 12 to 24 hours after the onset of the condition. It subsides when the inflammation disappears. A positive CRP may be present during the following conditions: pneumococcal pneumonia, streptococcal infections, rheumatic fever, lupus erythematosus, myocardial infarctions, and some kinds of carcinoma. It may also be present during sinusitis, pregnancy, use of oral contraceptives, and in women with an IUD in place. It is sometimes present in normal persons in the older age group.

Rheumatoid Arthritis Tests

The serum of patients with rheumatoid arthritis may contain a macroglobulin (protein) antibody called *rheumatoid factor (RF)*. This RF may be detected by its ability to agglutinate erythrocytes or latex particles during rapid slide testing. This is a nonspecific test that may also be positive in the following: lupus erythematosus, bacterial endocarditis, cirrhosis of the liver, viral hepatitis, and some forms of leukemia. Many different commercial kits are available for the detection of rheumatoid factor. You should follow the manufacturer's directions carefully. Additional testing, such as serial dilutions or ANA (Antinuclear Antibodies), may be required to aid in differentiating specific diseases.

Infectious Mononucleosis Tests

Infectious mononucleosis is a disease characterized by fever and an enlargement of the lymph nodes and spleen. Atypical lymphocytes and heterophil antibodies are found in the blood. In the laboratory, the technologist will distinguish the atypical lymphocytes from the normal lymphocytes and monocytes, and record the percentage of atypical cells as the differential cell count is done.

Heterophil antibodies differ from other antibodies in that they are nonspecific. For example, these antibodies in human blood serum may react with the red blood cells of sheep, causing them to agglutinate.

There are various types of tests for heterophil antibodies. A tube test consists of a series of dilutions of the patient's serum mixed in test tubes and incubated with washed sheep cells. Agglutination of the sheep cells indicates the presence of heterophil antibodies. The greatest dilution that reacts is reported.

This is a nonspecific test and therefore presumptive. The increase in serum agglutinins may be either from infectious mononucleosis or from serum sickness. Further tests that are specified for infectious mononucleosis should be done for differentiation.

The spot (**Mono-Test**, from Wampole Laboratories) is an example of a modified rapid differential test for infectious mononucleosis. It

uses a slide instead of test tubes. The following materials and procedure may be used to perform the Mono-Test.

You will need these materials:

- Centrifuge
- Control sera
- Calibrated capillary tubes and bulbs
- Glass slide (disposable card slide)
- Stirrers
- Mono-Test Reagent

Follow this procedure:

1. Centrifuge the patient's specimen (do not use heparin during collection of blood).
2. Fill capillary tube up to the mark with patient's serum.
3. Empty contents of tube into center of the middle section of the slide.
4. Place one drop of positive control serum on the left section of the slide.
5. Place one drop of negative control serum on the right section of the slide.
6. Add one drop of Mono-Test Reagent to each section of the slide.
7. Using a separate stirrer for each section, mix serum and reagent.
8. Spread over an area approximately 1 inch in diameter.
9. Observe for agglutination.

Use a high-intensity lamp for lateral illumination, and observe against a dark background. If using disposable cards:

1. Rock gently for 1 minute.
2. Leave undisturbed for another minute.
3. Observe immediately for agglutination.

Report as positive if agglutination of the Mono-Test Reagent occurs. Report as negative if there is no agglutination, or if the agglutination is of a finely granular pattern.

Lupus Erythematosus Tests

Lupus erythematosus (LE) is a chronic disease that affects the connecting substance of the blood vessels. Besides fever, arthritis, and internal organ involvement, there is a butterfly-shaped rash that appears across the bridge of the nose and face. An antibody found in affected serum is designated the *LE factor.* The LE factor stimulates the formation of a characteristic leukocyte called the lupus erythematosus cell *(LE cell).* These LE cells phagocytize the nuclear material of other leukocytes. The detection of LE cells is useful in diagnosing the disease.

An LE cell test requires a blood clot containing the LE factor, damaged leukocytes, and normal leukocytes. When all these factors are present, LE cells may be formed. Blood is collected from the patient and is allowed to clot undisturbed for 2 hours. The clot is then macerated with a pestle and strained through a sieve. This is then put into small-bore tubes, which are thoroughly centrifuged. The hemolyzed liquid upper portion is discarded. Smears are allowed to dry and are then stained with regular blood stain. The classic LE cell contains a purplish, round mass that is the remnant of the nucleus of a phagocytized cell. The mass has a velvet-textured appearance. Smears are examined microscopically and are reported as "LE cells present" or "no LE cells seen."

Alternate Procedure for LE Preparation

Perform a venipuncture to collect blood in the 10 ml special Vacutainer containing 10 heparin-coated glass beads. Immediately rotate 15 to 20 times. Allow to set at room temperature for 30 minutes. Place on the automatic rotator for 30 minutes, then allow to set at room temperature for 1 hour. Centrifuge at three-fourths speed (about 3,500 rpm) for 10 minutes, and discard plasma. Using a disposable glass Pasteur pipette, pipette off the buffy coat and a few red blood cells. Place these in a Wintrobe sed rate tube and centrifuge at 3,500 rpm for 10 minutes. Prepare four to eight smears on glass slides from the buffy coat after centrifugation. Stain with Wright's stain, and observe for LE cells.

Several other tests for lupus erythematosus have been devised by various manufacturers. It is wise to check your local laboratory and see what procedure is used there.

Febrile Agglutinations Tests

When a patient continues to exhibit fever for unknown reasons, a battery of tests known as "febrile agglutinations" may be ordered to determine the cause of the Fever of Undetermined Origin (F.U.O.). This test battery is used to differentiate among typhoid fever, paratyphoid fever, tularemia, undulant fever (brucellosis), and the rickettsial diseases; typhus fever, trench fever, and Rocky Mountain spotted fever. The test detects antibodies in the patient's serum. The antigens used in testing are prepared from the specific bacteria that cause the various diseases. For example, the antigen used to test for typhoid fever is prepared from *B. typhi O* or *B. typhi H. B. paratyphoid (A, B, or C)* is used to test for paratyphoid fever. *F. tularensis* is used to test for tularemia, and *Brucella* is used to test for brucellosis. A nonspecific antigen using *Proteus OX-19, OX-K, and OS-2* is used to test for the rickettsial diseases. These antigens are available commercially.

The procedure for febrile agglutinations includes the following materials:

- Pipettes to deliver serum
- Febrile-type, glass-ringed slides (five rings across, six rings down)
- Centrifuge
- Stirrers
- Commercial antigens
- Control sera

Then follow these steps:

1. Centrifuge blood to separate serum from cells.
2. Using special febrile pipette, deliver 0.08, 0.04, 0.02, 0.01, and 0.005 ml serum to the top row of rings across the slide.
3. Repeat procedure to the second row, and each subsequent row until all six rows have serum (see fig. 3).
4. Be certain to prepare control serum in the same amounts.
5. Add a drop of the commercially prepared antigen to each amount of serum all the way across the slide. Start at the right side of the slide.
6. Use the stirrer to thoroughly mix each ring.
7. Use a different antigen for each cross row.
8. When all rings are mixed, rotate the slide 20 times by hand.
9. Read immediately for agglutination.
10. Agglutinations of 2 + + are reported.
11. Dilutions for the rings are 1:10, 1:20, 1:40, 1:80, and 1:160. If the last ring is positive (1:160), you must make further dilutions and repeat the procedure.

When testing with the *F. tularensis* antigen, place the slide on a mechanical rotator at 150 rpm for 4 minutes. Read immediately.

Your laboratory may prefer to check only the 1:10 dilutions (0.08 ml serum) before setting up the entire slide.

One reactive test may not be significant and should be repeated. A rising titer 3 to 5 days apart is significant.

Antistreptolysin O (ASO) Test

In 90 percent of streptococcal infections, the body produces specific antibodies in response to extracellular products (Streptolysin O antigens) of Group A Streptococcus. In the body,

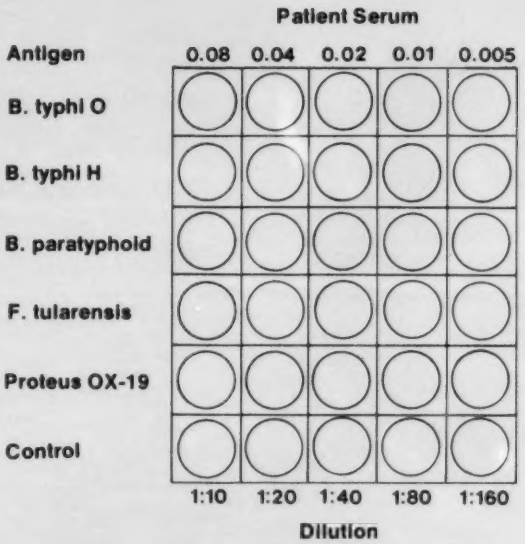


Fig. 3. Testing for febrile agglutinations using glass-ringed slide

Streptolysin O antigen produces lysis of red cells (i.e., flushed skin color in scarlet fever). Titers begin to rise about 7 days after the onset of the infection and reach maximal levels after 4 to 6 weeks.

Since there are several modifications of the ASO test, you should follow the manufacturer's instructions for the test used in your laboratory. An elevated ASO titer is helpful in diagnosis of recent Group A Streptococci infection, acute rheumatic fever, and acute glomerular nephritis.

Cold Agglutinins Test

Many normal people have cold agglutinins in the blood. These are agglutinins that will cause the person's own red blood cells to agglutinate at temperatures between 0° and 5°C. However, even if cold agglutinins are present, they are usually demonstrable only in undiluted or slightly diluted serum. The agglutination is reversible. It breaks up on warming and occurs again upon chilling. The agglutinin can be adsorbed onto red blood cells in cold temperature, thus removing them from the serum. This procedure must often be done in blood banking to achieve a compatible cross match. However, cold agglutination is of most clinical significance in confirming the diagnosis of viral (atypical) pneumonia.

The following procedure may be used to test for cold agglutinins:

1. Collect a tube of venous blood.
2. Immediately place blood in 37°C water bath for 1 hour.
3. Allow to clot undisturbed during incubation.
4. Centrifuge and remove serum.
5. Prepare mixture of red blood cells.
6. Wash three times in physiological saline.
7. Prepare 2% suspension of washed red cells (0.2 ml cells diluted to 10 ml with saline).
8. Make serial dilutions of patient's serum.
   a. Place 0.2 ml saline in tubes labeled #1 through #10.
   b. Add 0.2 ml of serum to tube #1, and mix thoroughly.
   c. Transfer 0.2 ml of contents of tube #1 to tube #2. Mix thoroughly.
   d. Transfer 0.2 ml of contents of tube #2 to tube #3, and continue through tube #9.
   e. Discard 0.2 ml from tube #9.
   f. Tube #10 is the control and contains only saline.
9. Final dilutions of serum are 1:2, 1:4, etc.
10. Add 0.2 ml of 2% cell suspension to each tube.
11. Shake rack to mix.
12. Refrigerate overnight.
13. Read agglutination of 1+ or more, and report as the reciprocal of the highest dilution that shows agglutination.
14. Reversibility is demonstrated by placing in a 37°C water bath for an hour.

Coombs Test (Antiglobulin Test)

The Coombs is a nonspecific test used to detect weak or incomplete antibodies. It was first used to demonstrate weak Rh agglutinins. The Indirect Coombs Test detects antibodies *in vitro*—antibodies in blood serum. The test is done routinely in Blood Banking. Sometimes incomplete antibodies are not detected in the routine cross-matching procedures. The Indirect Coombs Test detects the presence of incomplete antibodies that might result in incompatability. This test is also done routinely on new obstetrical patients and then periodically on Rh-negative obstetrical patients.

The Direct Coombs Test detects antibodies *in vivo*—antibodies attached to the red blood cells. In this test, the patient's red blood cells are mixed with antihuman serum. The antihuman serum is usually prepared from the blood of rabbits that have been injected with human red blood cells. Agglutination of the patient's red cells is a positive test. The Direct Coombs Test is used for the detection of hemolytic disease of the newborn, autoimmune hemolytic anemia, and in the investigation of hemolytic transfusion reactions.

Tabletop Centrifuges and Their Calibration

Centrifuges are perhaps among the most frequently used pieces of equipment in the clinical laboratory. They are used in urinalysis, hematology, chemistry, serology, and blood banking so much that it is often difficult to carry on conversations in a normal tone—especially if all the centrifuges happen to be in use at the same time. In this section, we will concentrate mainly on the tabletop centrifuges and their calibration. This is the type of centrifuge used in serology or blood banking.

Centrifuges are used in serological testing to improve red blood cell antigen-antibody reaction and also to wash cell suspensions. One type of centrifuge has fixed speed and variable time. The other has both variable speed and variable time. The routine checks of revolutions per minute (rpm) and time of centrifugation are absolutely essential for consistent results that meet quality-control standards.

Variable-speed centrifuges have a relative setting indicator as part of their speed control. To set the speed control, a tachometer is used to determine the number of revolutions it makes per minute. Then the speed is marked as such. From time to time the laboratory will reverse the procedure. The control is set, and the tachometer is read to see if the specified revolutions are actually being produced. Tachometers may be the strobe or vibrating-reed type.

Centrifugation time is checked with a stopwatch. Time is checked from the beginning of acceleration to the point of deceleration. Acceleration time is more difficult to control because it must be varied according to the thickness (viscosity) and volume of the suspending liquid through which the cells must pass in the tube. Therefore, it is necessary to calibrate the centrifuges for all the different kinds of tests that are performed.

Each centrifuge should be calibrated to determine the speed and time that produces agglutination without causing false positive reactions. Centrifugation causes the cells to come together in a well-defined button (delineated). However, centrifugation should not pack the cells so tightly that unagglutinated cells cannot be resuspended with gentle agitation. A clearly delineated cell button is a small round dot surrounded by clear supernatant fluid. The outer surface of the cell button should be clear and crisp, not jagged or fuzzy.

When the proper setting for centrifugation is determined for the kind of test to be performed, the supernatant fluid will be clear, the cell button will be clearly delineated, and it will resuspend with very gentle agitation. The final time that is selected must show the strongest reaction with the positive cell, as well as a clear-cut negative reaction with the negative cell. The final calibration selection should be marked on the centrifuge (along with the date the calibration was performed) so that everyone will use the proper settings.

To check the speed control using a *stroboscope,* put a transparent heavy plastic lid on machines that have metal covers. Place a mark on the centrifuge shaft, and start the motor. Turn the strobe light to "ON," and adjust until the mark appears to stand still. At this point, read the speed of rotation from the stroboscope dial. Check variable-speed centrifuges at several dial settings, both loaded and unloaded. Compare the speeds with those listed on the calibration chart for that centrifuge.

It is perhaps easier to check the speed control by using a vibrating-reed type tachometer because this simply attaches to or is held against the casing while the centrifuge is running. Reeds of various resonating frequencies are used in miniature tuning forks. Each one vibrates as the centrifuge reaches the revolutions needed to activate it. Record the speed of revolution as it is determined.

To *calibrate centrifuges,* follow this procedure. (1) Use serum that contains an antibody that will produce a 1+ agglutination when viewed with the unaided eye (macroscopically). Commercial antiserum may be diluted with bovine albumin or human group AB serum to produce a satisfactory protein concentration. Some laboratories use

anti-Rh_o (D) that is diluted about 1:25 for a 1+ reaction with Rh-positive cells. (2) Select a negative sample. Prepare a suspension of fresh red blood cells in the concentration that is used in routine testing (2%–5%).

A. *For saline-active antibodies:*

Serum for group A (anti-B) is diluted to produce 1+ agglutination.

Positive control: group B red cells in suspension.

Negative control: group A red cells in suspension.

B. *For albumin-active antibodies:*

Anti-Rh_o (D) is diluted to produce 1+ agglutination.

Positive control: Rh-positive red cells in suspension.

Negative control: Rh-negative red cells in suspension.

For each medium (saline, albumin, etc.), prepare five 10 × 75 or 12 × 75 mm tubes for positive reactions and the same for negative reactions. Add serum and test cells to each tube just before centrifugation. In pairs (a positive and a negative), centrifuge the tubes for different times (10, 15, 20, 30, 45, and 60 seconds). Observe each tube for agglutination, and record results as shown in table 1.

The optimum time of centrifugation is the least time required to fulfill all of the criteria. Since, in table 1, the 45-second and 60-second spins are both adequate, the optimum time for this centrifuge is 45 seconds.

Figure 4 shows an actual sample of a calibration record from a Texas hospital laboratory.

TABLE 1

	Time in Seconds					
Criteria	10	15	20	30	45	60
Clear supernatant	no	no	yes	yes	yes	yes
Delineated cell button	no	no	no	no	yes	yes
Cells easily resuspended	yes	yes	yes	yes	yes	yes
Negative reactions clear	yes	yes	yes	yes	yes	yes
Degree of agglutination	0	0	wk+	1+	1+	1+

Calibration Record

Centrifuge Serial No. CICCC

Saline Controls Anti A Date 1/19/81 Initials RAA

Positive A cells Negative C cells

Albumin Controls Anti D Date 1/19/81 Initials RAA

Positive Coccitive Cells Negative C NEGATIVE Cells

Results

	Times	Saline				Albumin				Enzyme				Coombs Wash				Coombs Spin			
		15	15	20	30	10	15	20	30												
1 Clear Supernate?	Pos Control	No	No	Yes	Yes	Yes	Yes	Yes	Yes												
	Neg Control	No	No	Yes	Yes	Yes	Yes	Yes	Yes												
2 Delineated Cell Button?	Pos Control	Yes	Yes	Yes	Yes	No	No	No	Yes												
	Neg Control	Yes	Yes	Yes	Yes	No	No	No	Yes												
3 Easy Resuspension?	Pos Control	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes												
	Neg Control	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes												
4 Strength of Agglutination	Pos Control	Neg	Neg	Wk+	1+	0	1+	1+	2+												
	Neg Control	Neg	Neg	Neg	Neg																
Optimum Time		30 sec.				30 sec.															

R.P.M. Check

Date	Method	Reading	Tech
8/8/80	Vibrating Reed	3500	RAA
1/19/81	Vibrating Reed	3500	CP

Timer Check

Date	Method	15	30	45	60	120	240
8/8/80	Stopwatch	-	-	-	-	-	-
1/19/81	Stopwatch	-	-	-	-	-	

Maintenance Record

Date	Work done	Tech
1/19/81	cleaned centrifuge head	CP

47

54

Fig. 4. Calibration record

55

UNIT 6

Bacteriology

Objectives: After completing this unit, you will be able to–

1. describe the procedure for collection of specimens;
2. prepare media;
3. discuss the relation between sterilization time and temperature/pressure;
4. inoculate, streak, and store cultures properly;
5. explain the use of CO_2 atmosphere;
6. apply antibiotic susceptibility discs;
7. perform Gram stain;
8. perform acid-fast stain;
9. collect skin scraping for fungus study; and
10. understand the importance of media inventory.

New Terms and Definitions

aerobic–grow in the presence of free oxygen.

agar–substance used in culture media; liquid when warmed, solid at room temperature.

anaerobic–grow only if there is no oxygen.

aseptic–free of microorganisms.

autoclave–sterilizers designed to use steam as the sterilizing agent.

bacteriology–the study of bacteria.

contaminant–foreign organism developing accidentally in a culture.

dermatophyte–fungus parasite on the skin.

fix–to fasten or hold firm and not wash off with water or staining solution.

inoculate–the introduction of microorganisms onto culture medium.

inoculating loop/needle–device used to transfer specimens or microorganisms from one medium to another. Once transfer has been accomplished, this is used to streak (spread) the material (inoculum) that was transferred.

medium–substance used to provide nutrients for the growth and multiplication of microorganisms (plural is *media*).

microbiology–study of living organisms that can be seen only under a microscope.

mycelia–threadlike filaments that constitute the vegetative structure of a fungus (Hypha is a single filament or thread of a mycelium).

normal flora–organisms normally present and for some reason able to resist the defensive mechanisms of the person but at the same time unable to invade body tissues and cause disease (unless the person's resistance is lowered).

optimum-growth temperature–temperature of incubation that gives the most rapid growth during a short period of time (12 to 24 hours).

pathogenic–capable of producing disease.

petri dish (plate)–shallow, round dish made of glass or plastic with a cover. Used to hold solid media for culturing bacteria.

sterilization–process of destruction of all microorganisms on a substance.

sterilizing agent—that which causes the death of all bacterial life.

susceptibility testing—placement of discs containing antibiotics onto culture of microorganisms to determine which antibiotics would prevent or retard growth of that microorganism.

symbiosis—living together of two or more organisms in a more or less mutually beneficial state.

Definition of Microbiology

Defined simply, **bacteriology** is the study of bacteria. A broader and perhaps more appropriate term is microbiology. **Microbiology** is the study of living organisms that can only be seen under a microscope. These organisms include bacteria, yeast and molds, protozoa, and the viruses. A few large medical centers separate the microbiology department into strictly bacteriology (bacteria), mycology (fungi or yeast and molds), parasitology (parasites), and virology (viruses).

Microorganisms are found in large numbers on and in the human body. Under normal conditions, these microorganisms are harmless. They are called part of the normal flora (normally present and not pathogenic or disease-producing). Organisms that are part of the **normal flora** are those that for some reason are able to resist the defensive mechanisms of the person. At the same time, they are unable to invade the body tissues, unless the person's resistance is lowered. Therefore, the person and the bacteria (microorganisms) exist in a **symbiotic** (living together) relationship. Normal flora of one part of the body differ greatly from the normal flora of another part. But as long as the person's resistance is maintained at a sufficiently high level, the bacteria that make up the normal flora do no harm. However, from time to time, organisms invade tissues and become pathogenic. It is the responsibility of the microbiology (bacteriology) department to grow, isolate, and identify the microorganism that is causing the disease condition.

Collection of Specimens

Generally, there is a joint effort by the nursing staff and the laboratory personnel to obtain the proper specimen for analysis. In hospitals, nursing usually has the responsibility to collect the specimen, label it properly, and send it to the lab. In the clinic or physician's office, the medical laboratory personnel may often be part of the collection team. But no matter who the collection agent is, the specimens submitted for bacteriological examination must be collected by aseptic technique into appropriate sterile containers. The specimen must then be carried immediately to the laboratory.

It is important that specimens are **NOT** left sitting around the nursing station or the laboratory because the pathogenic microorganisms may become overgrown by contaminants. It is the responsibility of the laboratory personnel to see that the specimen is inoculated as quickly as possible onto the correct media. Generally, if a specimen cannot be processed within an hour or less after it is collected, it should be refrigerated to prevent contaminant overgrowth.

Quality control in the bacteriology laboratory most certainly begins with specimen collection. It then continues through logging, processing, cultivating, isolation and identification, susceptibility testing, and reporting.

Here are some general guidelines to be observed in the collection of specimens:

1. Stool specimens should be collected in clean cartons and carried immediately to the lab for culture and/or ova and parasite examination. If a delay is necessary, a preservative such as 0.033 M phosphate buffer and glycerol should be added to ensure survival of *Shigella* and *Salmonella* bacteria. The use of 10% formalin and PVA will preserve trophozoites and cysts of protozoa as well as eggs, larvae, and small adult worms.
2. Rectal swabs should be placed in GN broth.
3. Swabs for *Neisseria gonorrhoeae* should be inoculated on Thayer-Martin or Transgrow **at the time of collection.**

4. Aspirates and washings should be collected into sterile containers and promptly delivered to the lab. They should be processed immediately to ensure recovery of the pathogens and prevent overgrowth of contaminants.
5. Spinal fluid (CFS) must be collected into a sterile screw cap tube and sent immediately to the lab. This is already an excellent medium for bacterial growth, and it should **not** be refrigerated.
6. Blood specimens should be collected from a venipuncture site that has been properly disinfected with alcohol and iodine. The blood should be collected into one bottle of SPS medium that has an anaerobic atmosphere. Some blood should also be collected into one bottle of SPS medium that has an aerobic atmosphere.
7. Urine should be either a catheterized or a midstream, clean catch specimen collected after proper cleansing of the genital area. A sterile, wide-mouth container should be used. Specimens should be processed within 2 hours.
8. Swabs from wounds and abscesses suspected of having anaerobes should be placed in an anaerobic transport unit (prereduced swabs or evacuated. anaerobic glass tubes).
9. A swab of a body part for aerobes and yeast should be placed in a sterile transport medium and delivered to the lab.
10. Biopsy specimens should be collected aseptically in a sterile container with sterile saline or phosphate buffer added to prevent drying.
11. Skin, hair, or nail clippings should be collected in sterile petri dishes or tubes.
12. Generally, if a specimen cannot be processed within an hour, it should be refrigerated to prevent overgrowth by contaminants.

Preparation of Media

Because of convenience, availability, and economics, most laboratories no longer routinely prepare their own media. This has been a tremendous help to the bacteriologist, the physician, and the patient. The bacteriologist can now spend time on isolation and identification that used to be spent in mixing, sterilizing, pouring, and storing media. Media are purchased preprepared, wrapped, labeled, and packaged ready to be stored in a variety of positions. The quality of the commercially prepared media is always the same (when stored properly). This has not only helped the patient, but also cut identification time and susceptibility testing time in half or more. There may be times, however, when there is a delay in shipment or a greater use of media than expected, and the lab has to prepare media to "tide them over until the shipment arrives." The medical laboratory student should have at least a basic appreciation for media preparation.

All media to be prepared come in containers that have specific instructions in a conspicuous place. *Follow the manufacturer's instructions exactly!* All glassware must be clean. After the media have been properly mixed, they must be sterilized. It does no good to inoculate the patient's specimen onto media that are not sterile. You would not know whether the bacteria that grow and multiply on the media were from the patient or from the media itself.

All biological systems, from bacteria (and viruses) to humans, have certain nutritional requirements. Bacteria require such things as (1) energy source, (2) carbon, (3) nitrogen, (4) several metallic elements, (5) certain vitamins and vitaminlike compounds, and (6) water.

To positively identify bacteria, several different types of media may be required. Nutrient media, enriched media, selective media, differential media, assay media, media for counting bacteria, and media for characterization of bacteria may be prepared according to manufacturer's directions. Generally, media are prepared as follows:

1. Place the required ingredients or dehydrated powder into a mixing vessel (usually a flask).
2. Add half the required volume of water, and mix thoroughly. If the medium con-

tains agar, allow it to soak for 5 minutes. Add the remainder of the water.

3. If heat is necessary to dissolve the ingredients, heat gently and mix frequently (stirring rod or swirling) until boiling. Boil for 1 or 2 minutes. *NOTE:* Some media are **never** to be boiled or autoclaved. Follow manufacturer's instructions!
4. Adjust pH if necessary.
5. Pour the media into containers (tubes, bottles, or flasks), and close with cotton plugs or screw caps (loosely tightened).
6. Sterilize (*except* those that are not to be sterilized!).

Sterilization

Sterilization is the process of destruction of all microorganisms on a substance, including all vegetative forms. To obtain sterilization, every microorganism and spore must be destroyed. There is no such condition as "almost sterile" or "nearly sterile." Either an object is sterile, or it is not.

Sterilizing agents are those which cause the death of all bacterial life. Sterilizing agents in use today include steam, dry heat, ethylene oxide and other chemicals, and high-energy electron beams or gamma radiation.

Sterilizers designed to use steam as the sterilizing agent are called **autoclaves** to distinguish them from sterilizers that use agents other than steam. In an autoclave, the steam is under pressure in order to obtain high temperatures. Fifteen minutes at 15 pounds of pressure (which is 121°C) is sufficient to kill all bacterial life that would be found in media. There are times when you should not use temperatures as high as 121°C. Media that contain sugars, for instance, should be autoclaved at 10 minutes and 10 pounds of pressure (which is 100°C). There are also times when you would use increased time and temperature to achieve sterilization. For example, after a specimen has been inoculated, the culture has been processed, cultivated, isolated, and so on, you would destroy the culture by autoclaving it at 50 minutes and 15 pounds of pressure (121°C) or 30 minutes at 132°C. Then the old discarded cultures are placed in plastic bags and put into the trash. **Never** discard cultures until they have been autoclaved to destroy all the bacterial life.

Processing Specimens

At this point, the media have been prepared (either commercially or by laboratory personnel), and sterilization procedures have been performed (on media prepared in the lab). The bacteriologist can now begin to process the specimens. Specimens are sent to the lab on swabs that have been collected aseptically. The specimen must be labeled properly with the patient's name, room number, identification number, physician's name, date and time of collection, and exact source of the specimen. "Wound," "abscess," etc., are not complete sources. If the wound is on the "left side of the kneecap," that is what should be reported as "source."

After specimen identification is certain, the specimen information should be written into the log book. A lab number for identification purposes is given to each specimen. Select the media that are appropriate for the type of specimen, streak the petri dishes of media, and prepare smears for staining. Then place the inoculated plates into the incubator upside down.

Bacteriological Procedures

There is an art to streaking plates. Few people do it perfectly on their first attempt. We will discuss the **streak plate technique** for specimens that reach the lab on swabs and for urines for culture. It is important to use the entire plate of medium. The entire procedure should be performed aseptically.

Flame the inoculating loop/needle before and after using, between streaks of a different direction (as when streaking plates for isolation), and, of course, between specimens. This is to prevent contamination. The inoculating loop/needle should also be flamed before introducing it into any container and after the specimen has been obtained.

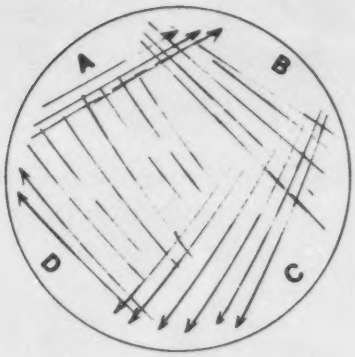


Fig. 1. The streak plate technique

Remove the container top, and quickly pass the mouth of the container through the flame to destroy any contaminants on the outside of the container. Remove the swab, and inoculate the medium by rolling the swab along one edge of the petri dish (see fig. 1A). Place the swab back into the container again, and replace stopper. Flame an inoculating needle (piece of wire without a loop) inserted in a holder device. Many labs use a Bunsen burner type of apparatus. Other labs use an electric burner device. Heat the needle (or loop) to redness in the flame (or heater). Touch to the side of the medium to cool, and streak across the area where you inoculated the medium (fig. 1B). Flame the needle again, and streak across the tip (fig. 1C) you just finished streaking. Flame and repeat (fig. 1D). Close the petri dish, flame the needle, and return it to the usual storage place. Be certain the petri plates are all labeled. Place in incubator at proper carbon dioxide (CO_2) atmosphere.

If the specimen is urine (or other liquid), use an **inoculating loop.** Most loops are calibrated to contain a certain amount. Other loops may not be calibrated. Follow the procedure for your training station.

Flame the mouth of the container to destroy any contaminants on the outside. Flame the loop to redness, touch the loop to the top of the liquid to cool, and then on the liquid to be transferred. Transfer the loopful to the medium, and streak the loop down the center of the petri dish (see fig. 2). Flame the loop, touch it to the medium to cool, and then streak across the entire top half of plate. Turn the plate around, and repeat until the entire plate is streaked.

Consult lab procedure chart or manual to determine which cultures are grown in oxygen on the incubator shelves and which must be placed under reduced or no oxygen to obtain optimum growth. Bacteria fall into four categories (see fig. 3). *Aerobic* bacteria grow in the presence of free oxygen. *Anaerobic* bacteria grow only if there is no oxygen. *Facultative anaerobes* grow in either the absence or presence of free oxygen. *Microaerophilic* bacteria grow if only very small amounts of free oxygen are present.

The **cultivation of anaerobes** requires special attention and equipment. The equipment may be as simple as a candle jar. This is a jar with a tight-fitting lid, large enough to hold a petri dish and a candle. The culture is placed in the jar, the candle is lighted, and the jar lid is fastened securely. As the candle burns out, an atmosphere containing 3 to 5% carbon dioxide (CO_2) results.

Elaborate, sophisticated and expensive CO_2 **incubators** that strictly control the amount of oxygen and carbon dioxide present are used in larger laboratories. The CO_2 incubator allows air and CO_2 to enter. Controls allow the mixing

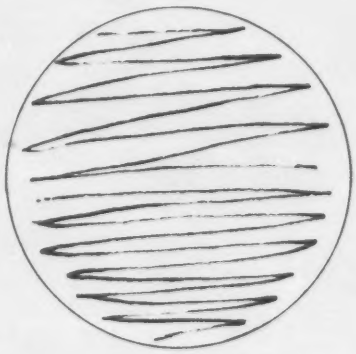


Fig. 2. Using an inoculating loop

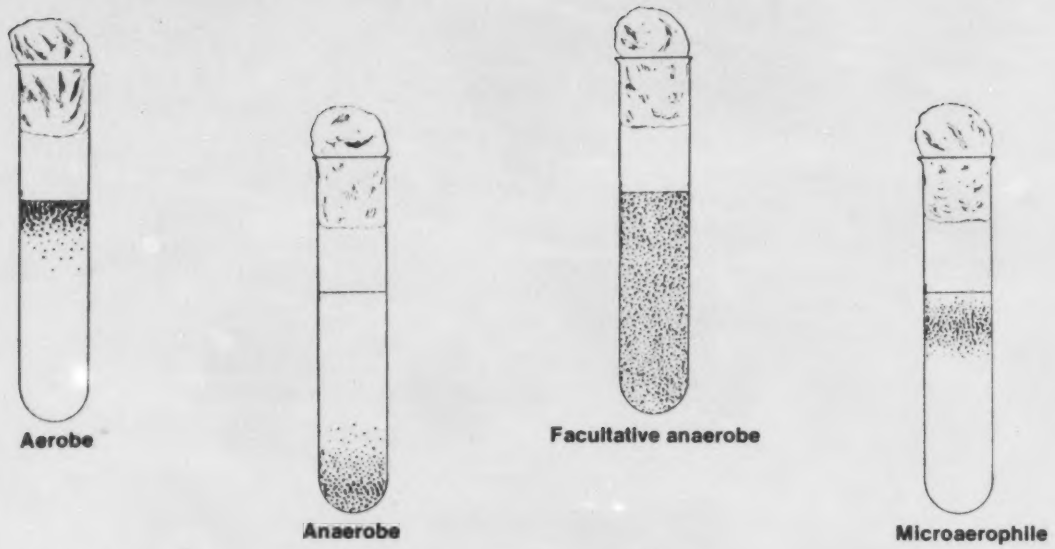


Fig. 3. Oxygen requirements of bacteria

of the air flow and pure CO_2 to achieve the ideal 5 to 10% CO_2 content.

If only reduced oxygen content is desired, BBL (Baltimore Biological Laboratories) has a commercial **gas-pack** available. Simply add the required amount of water to the pack, place the petri dish and the gas-pack into a jar, close the jar, place in incubator, and CO_2 will be released.

For those bacteria that are strict anaerobes, a gas-pack is available that has an indicator which is blue when oxygen is present and is white when the anaerobic condition has been attained. Again the petri dishes and the special gas-packs are placed into an anaerobic jar, and then into the incubator for optimum growth at the desired temperature.

Most pathogenic microorganisms grow well at body temperature (around 35°C). The *optimum-growth temperature* is the temperature of incubation that gives the most rapid growth during a short period of time (12 to 24 hours).

The culture is allowed to grow for 12 to 24 hours before checking. If growth has occurred, identification may or may not be possible by sight or even smell. (The medical laboratory student is advised **NOT** to smell the cultures.)

If a **susceptibility report** is requested (see fig. 4), further growth is necessary. Isolated colonies are transferred to a large petri dish of nutrient agar. The entire plate is streaked heavily. *Antibiotic discs* are then placed onto the surface of the streaked agar plate. Many laboratories have commercial dispensers for the antibiotic discs. The discs must be so firmly placed that they will not fall off when the petri dish is incubated upside down. All cultures on petri plates are incubated upside down so that the moisture that forms inside the plate will not fall onto the surface of the culture. Be sure to flame the instrument used to press the antibiotic discs to the agar surface to prevent contamination of the culture.

The following procedure is standard for **susceptibility testing:**

1. Select four or five colonies of the organism to be tested.
2. Transfer these colonies by touching the top of each colony successively with the

22 SUSCEPTIBILITY REPORT

Patient's name

Room # I.R.#

ORGANISM A:
ORGANISM B:
ORGANISM C:
ORGANISM D:
SPECIMEN SOURCE

PHYSICIAN ORDERING TEST
TO BE DONE
TIME - DATE COLLECTED INITIALS

REQUESTED BY INITIAL | TIME | AM PM | DATE | REPORT DATE & TECH.

BROAD SPECTRUM	C	D	A	B	C	D
AMPICILLIN	3					
CARBENICILLIN	5					
CEPHALOTHIN	7					
CHLORAMPHENICOL	9					
GARAMYCIN	11					
KANAMYCIN	13					
NEOMYCIN	15					
PENICILLIN G	17					
STREPTOMYCIN	19					
TETRACYCLINE	21					

GRAM NEGATIVES	C	D	A	B	C	D
COLISTIN	23					
GANTRISIN	25					
NALIDIXIC ACID	27					
NITROFURANTOIN	29					
POLYMYXIN B	31					
SXT-TMP	33					

GRAM POSITIVES	C	D	A	B	C	D
BACITRACIN	35					
CLINDAMYCIN	37					
ERYTHROMYCIN	39					
METHICILLIN	41					

SUSCEP (S) | INTERMEDIATE (I) | RESISTANT (R)

LABORATORY

Fig. 4. Susceptibility report form

same loop to a tube containing 3 to 5 ml Mueller-Hinton or trypticase soy broth.

3. Incubate the tube at 35°C long enough (2 to 8 hours) to produce an organism suspension with moderate cloudiness.
4. Dilute the broth culture with sterile saline or broth to obtain a turbidity that matches a prepared standard.
5. Dip a sterile cotton swab into the adjusted inoculum.
6. Lift the swab above the fluid level, and rotate the swab against the inside of the tube to remove as much excess inoculum as possible.
7. Streak the entire agar surface with the swab evenly in three directions.
8. Allow the inoculated plate to dry for 3 to 5 minutes with the plate closed.
9. Place discs on the agar with a dispenser or sterile forceps. (With sterile forceps, press discs down gently on the agar to ensure even contact.)
10. Incubate plates immediately at 35°C.
11. Do not incubate in a candle jar.
12. Read plates after 16 to 24 hours of incubation.

A very important part of the bacteriological study is the **Gram stain.** Bacteria are differentiated according to whether they are *Gram-positive* (stain blue) or *Gram-negative* (stain red). Examination of smears stained by this technique reveals both the morphology and the Gram reaction of the bacteria. In the staining technique, the bacteria are treated with the following solutions: crystal violet, iodine solution, alcohol (decolorizing solution), and safranin (or some other red counterstain). All bacteria take up the crystal violet. The iodine fixes the stain to the Gram-positive bacteria. This keeps the stain from washing off when the decolorizing solution is added. When the counterstain is added, the Gram-positive organisms do not stain red since they are already stained. The Gram-negative bacteria lose the blue color during the decolorizing process, so they stain red. The procedure below, or some modification (follow manufacturer's instructions), should give a satisfactory Gram stain:

1. Prepare the slide with the smear, and allow to air dry. Then "fix" the smear to the slide by passing through a flame. (*CAUTION:* do not overheat.)

2. Pour crystal violet onto fixed smear for 1 minute.
3. Wash with tap water.
4. Pour iodine solution onto smear for 1 minute.
5. Wash with tap water, and remove excess water by blotting or shaking.
6. Decolorize 30 seconds with alcohol.
7. Blot dry.
8. Counterstain with safranin for 10 seconds.
9. Wash with tap water, and blot dry.

Your training station may vary the times somewhat, but the order is always the same.

Another staining procedure that is widely used is the **acid-fast stain,** which classifies bacteria into *acid-fast* and *non-acid-fast.* Acid-fast organisms contain a large amount of fatty and waxy substances in the cell. Once the dye penetrates the cell (assisted by heating), it is not easily removed by the decolorizing agent. All acid-fast bacteria are also Gram-positive. Follow this procedure for performing an acid-fast stain:

1. Cover fixed smear with carbol fuchsin, and gently heat the slide (slow steaming should occur) for 3 to 5 minutes.
2. Wash with tap water.
3. Decolorize with acid-alcohol until only a small amount of red dye remains.
4. Wash with tap water.
5. Counterstain with methylene blue for 30 seconds.

The acid-fast bacteria will appear red. The non-acid-fast bacteria will appear blue.

The patient with a *dermatophytic condition* (parasitic skin infection caused by a fungus) requires special attention. It is not always satisfactory to send a sterile swab to the laboratory after simply swabbing the skin area. The infected skin should be scraped with a sharp object such as a knife blade or glass slide. Care is taken to avoid injury to the patient.

The scrapings should be placed in a small amount of potassium hydroxide (KOH). This dissolves the debris and softens the epithelial cells so any fungus structures that are present can be easily observed.

A thorough search is made microscopically for hyphae, yeast, and spore forms. *Hyphae* are filamentous or threadlike pieces of a mycelium. *Yeast* are unicellular fungi that do not have mycelia. *Spores* are resistant, primitive, reproductive bodies.

Whether or not fungi are observed, a fungus culture should be prepared. Fungus cultures are different in that fungi grow both at room temperature and at 35°C in the incubator. Fungus cultures should be inoculated onto media such as Sabouraud dextrose agar. If contaminants are suspected, the addition of penicillin and streptomycin will prevent the growth of the contaminants but will allow the pathogenic fungi to grow. *All work with mycelia should be done under the hood to prevent breathing of spores.*

Typical fungus infections include ringworm, athlete's foot, barber's itch, monilia infections, and those causing systemic infections (affecting the body as a whole), such as blastomycosis, histoplasmosis, and coccidioidomycosis.

It is important that specimens for culture are inoculated onto the correct media. Most laboratories have a chart posted that lists the various sources of specimens and the different media to be inoculated. A limited list of media most often found in laboratories includes the following: Blood Agar, Nutrient Agar, Eosin Methylene Blue Agar (EMB), Thioglycollate Broth, Biggy Agar Slants, Chocolate Agar, Thayer-Martin Agar, MacConkey Agar, Salmonella-Shigella Agar (S.S.) Azide, Loeffler's, Bordet-Gengou, Sabouraud, and Petragnani.

Materials most commonly cultured are urine; spinal fluid; tissue fluids; stools; specimens collected from the nose, throat, wounds, abscesses, or genitalia; and materials suspected of containing fungi or acid-fast organisms.

Storing culture media in the refrigerator in a systematic manner and maintaining an inventory of the media on hand are very important tasks. The medical laboratory student may be assigned these duties. He/she must carefully analyze current and proposed needs to avoid

running out of media. If there is an unexpected need for culture media, or if a shipment is delayed, the student may be required to prepare media for immediate use from dehydrated stock.

Finally, here are some *general reminders* for bacteriology:

1. Rotate media during preparation. Shaking or vigorous stirring causes bubbles.
2. When pouring media into petri plates, leave the lid ajar for a few minutes to allow some moisture to escape.
3. Use aseptic technique in all phases of bacteriological work.
4. Store petri plates upside down to prevent collected moisture from falling onto colonies.
5. Refrigerate specimens that cannot be processed within an hour to prevent overgrowth of contaminants.
6. Flame inoculating needle/loop before inserting into specimen. Cool slightly before touching to specimen to prevent destruction of pathogens by heat.
7. Perform fungus studies under the hood to prevent breathing in of spores.
8. Always flame mouth of any container, such as a flask or tube, before introducing loop/needle and after specimen has been obtained.

UNIT 7

Chemistry

Objectives: After completing this unit, you will be able to–

1. select and use pipettes correctly;
2. prepare reagents following general instructions;
3. distinguish between hand chemistries and automated chemistry procedures;
4. list the parts of a photometer;
5. assist with quality control checks of equipment; and
6. list at least five general categories of test procedures.

New Terms and Definitions

accuracy–how close the obtained result is to the correct answer.

automation–development and use of instruments that can complete a testing process without any human intervention.

delineate–to outline in sharp, clear detail.

enzymes–biochemical catalysts that allow the body to carry out its chemical reactions at a moderate temperature and pH compatible with life.

mass–quantity of matter; often spoken of as *weight*.

mean–halfway between two extremes.

meniscus–curved surface of a liquid column.

molarity–concentration unit equal to the number of moles per liter of solution.

mole–molecular weight of a substance expressed in grams.

normality–a concentration unit equal to the number of equivalent weights per liter of solution. (This term is no longer used by those who prefer to use SI units–the international system of presenting measurements.)

pipette–calibrated glass tube open at both ends used to measure small volumes of liquid.

photometer–device that measures light.

range–the difference between the lowest and highest observations.

reproducibility–the ability to repeat a procedure time after time with exactly the same results.

serological pipette–a pipette that is graduated to measure various volumes within a given amount (e.g., 5 ml graduated in 0.10 ml).

shifts–results that are steady but on one side of the mean.

TC–marking on a pipette that indicates the pipette should be allowed to drain and is then rinsed with the diluent so the pipette will deliver the amount stated.

TD–marking on a pipette that indicates the pipette will deliver the stated amount when allowed to drain.

trends–values that go steadily up or steadily down several days in a row.

variability–when measurements or results disagree but are still within acceptable range.

volumetric pipette–a transfer pipette that measures a single amount (drainage with tip touching side–not blown).

Using Pipettes

The medical laboratory student is often required to perform measurements of mass and/or volume. To measure mass (less correctly spoken of as weight), balances of some type (double pan, single-pan, triple-beam, or analytical) are used. To measure volume, the student may use graduated cylinders, volumetric flasks, burets, and, most frequently, pipettes.

Pipettes are calibrated glass tubes open at both ends, used to measure small volumes of liquid. There are two general types of pipettes (see fig. 1). **Serological** (measuring) pipettes are graduated to measure various volumes within a given amount. These generally require filling to a calibration mark, then allowing to drain. They are calibrated in markings to yield within 1 to 2% of the stated volume when the last drop is blown out after draining has occurred. A 10 ml serological pipette is calibrated in either 0.1 or 0.2 ml. The amount is stated on the pipette as "10 ml in 1/10" or "10 ml in 2/10."

Volumetric pipettes, on the other hand, have only one or two calibration marks. If only one mark is present, the pipette is the TD type (to deliver). It should be allowed to drain down the side of the vessel or into the fluid to deliver the quantity stated on the pipette. If two calibration marks are present, TD and TC (to contain), the TD procedure is the same. Allow to drain down the side of the vessel or drain into the fluid. If the TC mark is used, allow the pipette to drain, then rinse with the diluent so the pipette will deliver the amount stated. Volumetric pipettes are often called *transfer* pipettes.

Glassware is calibrated at 20°C since liquids expand at high temperatures and contract at lower temperatures. Therefore, the temperature in the room where calibrated glassware is used should be about 20°C. Measurements should also be made at eye level to prevent distortion (see fig. 2).

Pipettes that are etched at the mouth end must have the last drop blown out. All others are allowed to drain naturally. If TC is marked, be certain to rinse the pipette in the diluting

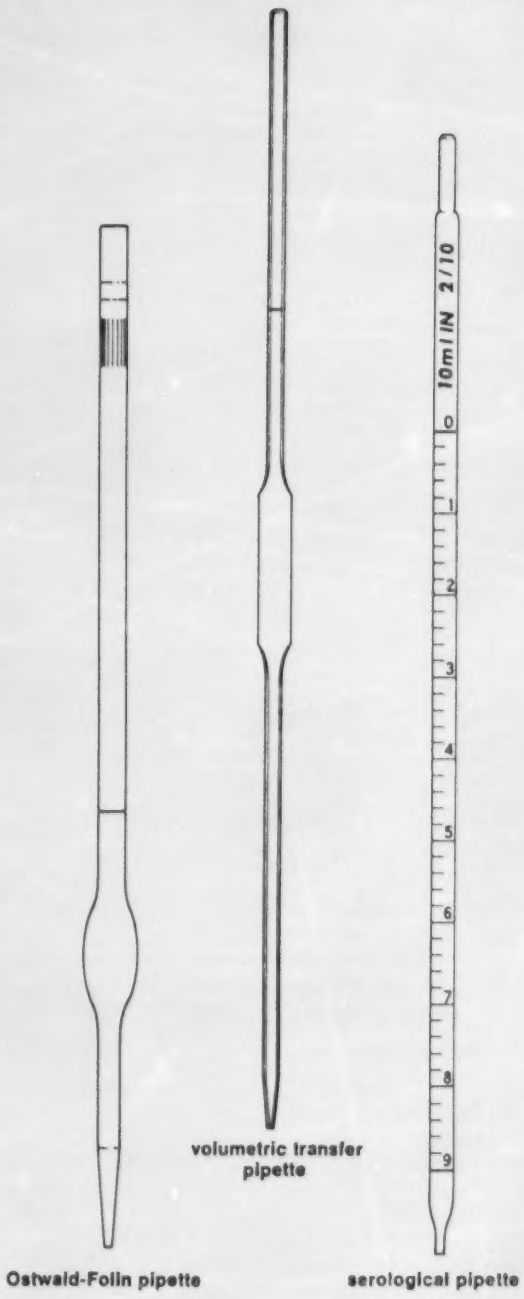


Fig. 1. Types of pipettes

solution so the amount left on the sides of the pipette will be insignificant. Pipettes must be cleaned with an effective cleaning solution. Then they are rinsed once thoroughly in tap water, then three times in distilled water. If it is ever necessary to use a pipette that has not dried thoroughly, rinse the pipette with the solution to be measured.

To use a pipette, place it into the solution to be measured, and use suction (like sipping on a soda straw) to draw the solution up above the desired mark. Place your tongue over the tip of the mouth end, and withdraw the pipette from your lips. Quickly replace the tip of the tongue with your index finger. Withdraw the pipette from the solution. Carefully allow the solution to drain until the bottom of the meniscus is on the desired calibration mark (see fig. 2). Place the tip of the pipette against the side of the receiving vessel, and allow the liquid to drain into it. If the top of the pipette is etched, blow out the last drop.

When pipetting concentrated acids and alkalis, deadly poisons, corrosive substances, and radioactive materials, use a mechanical suction device. **Do not use mouth suction for these.**

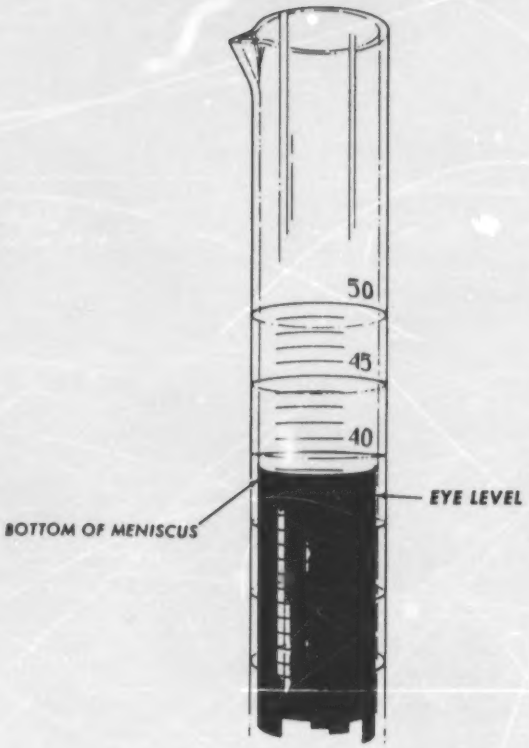


Fig. 2. Always read the bottom of the meniscus, at eye level.

Using Reagents

Most clinical laboratory reagents are purchased prepared and ready to use. However, if you are preparing reagents, you must remember certain precautions. For example, when working with acids, **always** add acid to water. **Never add water to acid.** Some acids, such as concentrated sulfuric acid, release large quantities of heat when diluted. Adding water to this acid will release enough heat to cause the water to boil and splatter and perhaps splash the acid on the medical laboratory assistant.

Most reagents are supplied as dry powder or crystalline powder. Each of these must be diluted in water. Generally, solutions are prepared by adding the required quantity of powdered reagent to a small amount of water. They are then carefully mixed to dissolve. Finally, enough water is added to bring the mixture to the final required volume. Always follow the manufacturer's directions on the original label for making solutions.

All prepared reagents including acids should be stored in the *original* container.

Manual and Automated Procedures

The medical laboratory assistant will find that there are many different acceptable procedures for laboratory tests. The procedures given here may be unlike those used in your laboratory. For example, simple blood analyses may be done manually, with the multisample analyzer (SMA), or using discrete glucose analyzers such as Clinicard or Beckman. Students must consult with their supervisor to find out which laboratory procedures they may actually perform entirely, which they may set up but must

have supervision to evaluate completely, and which they may only observe.

Automation is now used to some extent in almost all laboratories. **Automation** is the development and use of instruments to complete a testing process without human intervention. The automated system must include some kind of sampling device, a means to add the needed reagents and sample, incubating modules, modules to measure, and a recording device. The measuring module is usually a **colorimeter.** These same steps are followed in manual procedures. In fact, almost any manual method can be adapted to an automated system.

The human element is still vital in laboratory testing even though automation may seem to diminish the importance of the laboratory person. The technologist must still prepare serum, load sample cups, and, most important of all, be constantly aware of the validity of the automated system.

Spinal fluid analyses are still performed almost exclusively by manual methods. This is because the concentration of substances is very small, usually in milligrams. Most automated procedures are reported in grams.

Factors that determine the extent to which a given laboratory uses automated procedures are the size of the laboratory, economic feasibility, the number of tests performed, and the training and preference of the medical technologist.

Devices that measure light are called **photometers.** Figure 3 shows two basic types. Photometers are used in determining the presence and quantity of chemical substances in the blood. The substances in the blood can be treated in such a manner as to yield a color that can be measured quantitatively. *Visible* light is a result of energy changes of electrons in the outer shell. *Infrared* radiation is caused by vibrations of the molecules. *Ultraviolet* radiation is due to energy changes of electrons in the inner shells.

Photometers are classified according to the type of wavelength selector that is used. Wavelength selectors may be colored glass filters or diffraction grating (a thin piece of plastic ruled at regular intervals). Photometers must have a light source, a focusing lens, wavelength selector, photocell to convert the light to electricity, and a device to measure the electricity produced. The voltage supply to the light source must be constant.

The most important thing for the medical laboratory student to remember is to be certain the correct wavelength and selector are used for each test. The machine is set at Zero Absorbance (or 100% T) with the blank, and matched cuvettes (sample holders) are used. From readings of the spectrophotometer, calculations are made to determine the value of the sample that is tested.

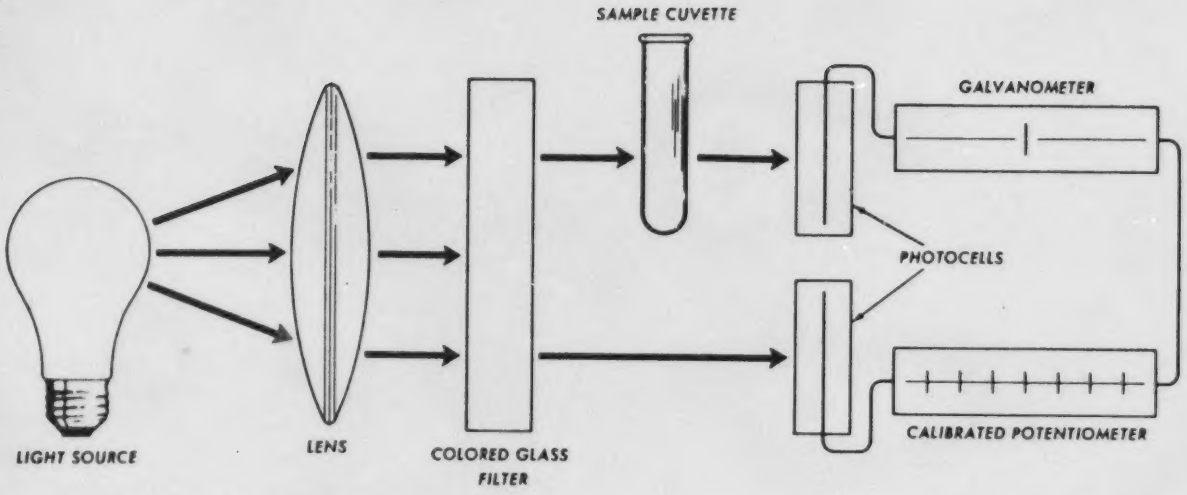
Quality Control

Any time a test result is reported, the technologist, physician, and patient want to be certain the report is correct. Today all laboratories are required by laboratory regulatory agencies to use some form of quality control to make sure that tests results fall within acceptable limits.

Quality control must exist at each step and starts with specimen collection. Careless work by any member of the laboratory team or malfunctioning of instruments can produce results that are unacceptable.

Equipment should be serviced and checked regulary. Water baths must have water at sufficient levels. Reagents must be checked to be sure they are fresh and uncontaminated. The temperature of water baths, heat blocks, and the refrigerator must be measured regularly. A complete and current record of these checks should be kept in an easily accessible place in the laboratory.

Control sera serve to check on temperature, pH, instrumentation, correctness of wavelength, reagents, technique, and possible deterioration of reagents and instrumentation. Control samples and standards must be run through regular test procedures. These results should be plotted. **Trends** or **shifts** over a period



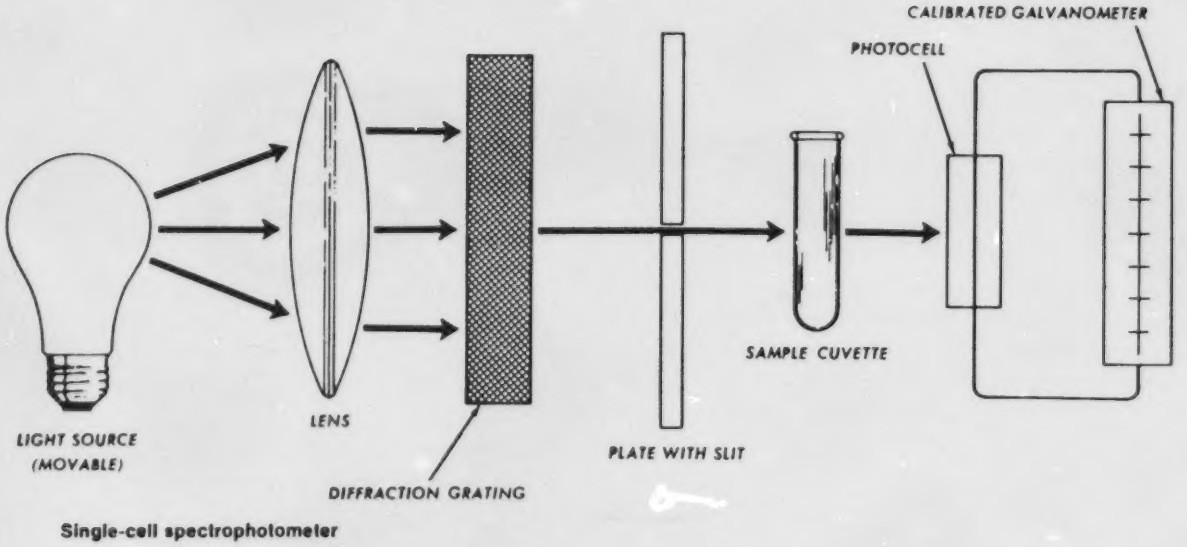


Fig. 3. Types of photometers

of several days can be easily spotted. By plotting controls, you can tell at a glance whether controls are within range. The **range** is the difference between the lowest and highest observations. The **mean** is halfway between two extremes. Quality control allows the laboratory to closely monitor its reproducibility, accuracy, and variability.

Quality control charts and data provide information that allows the laboratory to spot potential problems before patient results become meaningless to the physician. Quality control allows the laboratory to take immediate action before a test is completely out of control.

The medical laboratory student may be assigned the task of checking and recording temperature readings, water levels, and countless other checks that are a vital part of quality control. Figures 4, 5, and 6 are examples of quality control charts, sample charts, and maintenance charts.

General Tests

The general tests described here are routinely performed in the clinical chemistry laboratory.

Glucose or Blood Sugar. The oxidation of glucose provides energy for various biochemical processes. Diabetes mellitus is a disease that results from the body's inability to use glucose properly. Blood glucose determinations are used for diagnosis and treatment, as well as in routine physical examinations and health screening programs.

Blood Urea Nitrogen (B U N), Uric Acid, Creatine, and Creatinine. The kidneys excrete these chemicals as waste. An increase of these chemicals indicates that the kidneys are unable to excrete adequately.

Proteins (Albumin and Globulin). Proteins make up the support tissue of animals, protect us from disease in the form of antibodies, and are the basis for the basic functions of life. The ratio of albumin to globulin (A/G ratio) is used in the diagnosis of liver and kidney disease.

Bilirubin, Thymol Turbidity, Cephalin-Cholesterol Flocculation, and Bromsulfalein (BSP). These are all tests of liver function.

Triglycerides, Fatty Acids, Cholesterol, and Phospholipids Determinations. The first two serve as energy reserves. The latter are part of the structural units of cell membranes.

Calcium and Phosphorus. These are important in blood coagulation and kidney function. An increase in one results in a decrease in the other. A delicate balance must be maintained for proper functioning of body processes.

Electrolytes (Chloride, Sodium, Potassium, and Carbon Dioxide). Chloride is important in the maintenance of osmotic pressure and is the anion present in the largest amount in the body fluids. Sodium is the cation present in the largest amount in extracellular fluid. Potassium is the largest intracelluiar cation. These, along with CO_2, maintain a balance of the blood pH. The normal range of the blood pH is 7.35 to 7.45. This range is narrow, and deviations result in acidosis, alkalosis, or death.

Enzymes. Enzymes are biochemical catalysts that allow the body to carry out its chemical reactions at body temperature and at a pH compatible with life. Tests are most commonly done for the following enzymes:

- **Amylase** breaks down starches.
- **Lipase** breaks down fats.
- **Alkaline phosphatase** is important in the formation of bone and is elevated in obstructive jaundice.
- **Acid phosphatase** is elevated in cancer of the prostate, particularly if the cancer has spread to other parts of the body.
- **Transaminases** are elevated when there is deterioration in an organ.
  - *Serum glutamic oxalacetic transaminase (SGOT)* is present in heart muscle.
  - *Serum glutamic pyruvic transaminase (SGPT)* is found in liver tissue.
  - *Lactic dehydrogenase (LDH)* is used to diagnose cardiac and liver disorders.
  - *Creatine phosphokinase (CPK)* is used in diagnosis and prognosis following heart attacks.

Many laboratories are now equipped to perform *radioisotope studies.* These may be

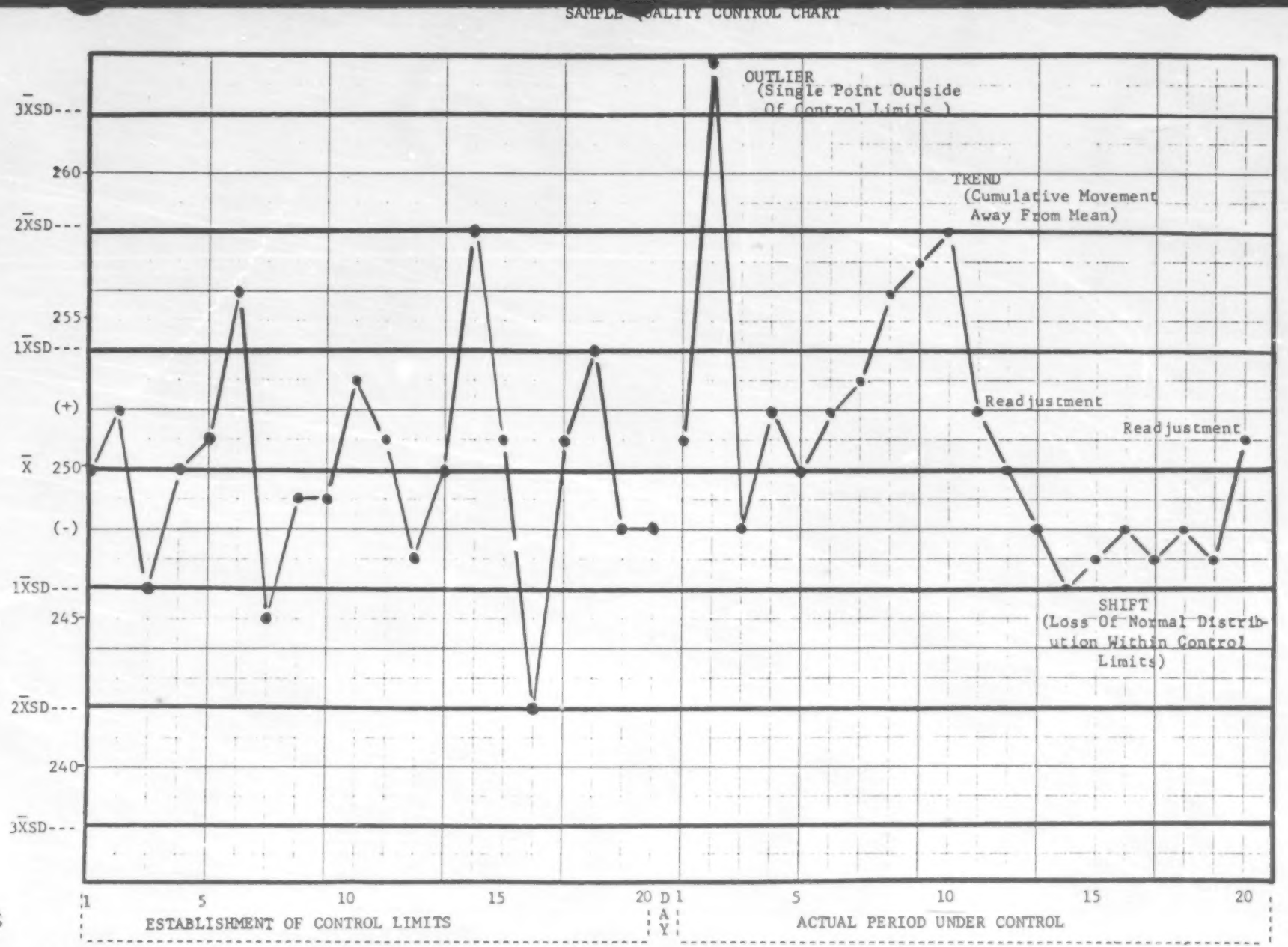


Fig. 4. Sample Quality Control Chart

MANUAL CHEMISTRY MAINTENANCE LOG

_____,19____

	Date / Initials		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	Prepare controls																																
2	Discard old specimen tubes (save day before only)																																
3	Check for refrigerated specimens																																
4	Clean counter space with 10% clorox																																
5	Check temperatures and record	37° waterbath																															
		37±1°/division	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Refrigerator (white)																															
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Freezer																															
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Perkin-Elmer																															
		37±1°/division	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Sensi-tex Incubator																															
		37±1°/division	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		Walk-in Refrig.																															
		5±2°/division	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	Empty water bottle in refrigerator as required																																
7	Check spectrophotometer absorbances	360 nm																															
		36±1%/division	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		572 nm																															
		11±1%/division	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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		620 nm																															
		98±1%/division	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Clean micro-centrifuge with 10% clorox																																
9	Clean 37° waterbath		X	X	X				X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X
10	Fill 37° waterbath (as required)																																
11	Clean centrifuges with 10% clorox																																
12	Replace centrifuge brushes (as required)																																
13	Clean work area with 10% clorox																																

Fig. 5. Manual Chemistry Maintenance Log

SPECIAL CHEMISTRY MAINTENANCE LOG

_____, 19____

			Date / Initials	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	Check temperatures		37° oven																															
			37±2°/division	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Refrigerator																															
			5±2°/division	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Freezer																															
			-3±2°/division	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			37° waterbath																															
			37±1°/division	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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			Corning 175																															
			37±0.2°/division	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Corning 165/2																															
			37±0.2°/division	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Clean 37° waterbath			X	X	X				X	X	X	X				X	X	X	X				X	X	X	X				X	X	X	X
3	Fill 37° waterbath																																	
4	Empty waste container		Corning 175																															
			Corning 165/2																															
5	Check liquid levels		175 Wash																															
			165/2 Wash																															
			175 pH Buffers																															
6	Check gas pressures		Corning 175																															
			Corning 165/2																															
7	Drain and fill pH reference electrode (165/2)																																	
8	Drain and fill pH reference electrode (175)			X	X	X				X	X	X	X				X	X	X	X				X	X	X	X				X	X	X	X
9	Replace membranes (pH, PO_2, PCO_2)		Corning 175	X	X	X				X	X	X	X				X	X	X	X				X	X	X	X				X	X	X	X
			Corning 165/2	X	X	X				X	X	X	X				X	X	X	X				X	X	X	X				X	X	X	X
10	Check manometer readings (175) see page 2, line 21																																	
11	Insert KOH		Corning 175	X	X	X				X	X	X	X				X	X	X	X				X	X	X	X				X	X	X	X
			Corning 165/2	X	X	X				X	X	X	X				X	X	X	X				X	X	X	X				X	X	X	X
12	Insert ECS		Corning 175																															
			Corning 165/2																															
13	Check gas flow rates		Corning 175																															
			Corning 165/2																															
14	Check barometric pressure and adjust if necessary (175)			X	X	X				X	X	X	X				X	X	X	X				X	X	X	X				X	X	X	X

Fig. 6. Special Chemistry Maintenance Log

used to test for thyroid function and even to check for pregnancy. Some laboratories perform Digoxin procedures.

Large reference laboratories continue to thrive. Many test procedures are ordered so infrequently that it would not be economically feasible to have on hand at all times everything necessary to perform the procedure. These tests are often sent to reference laboratories: ANA, CEA, and RIA studies of all kinds. Others are Digoxin, Dilantin, Folic Acid and B_{12} requests. Hepatitis B Antigen, Insulin, Serum Iron and Iron-Binding Capacity, Hormone studies, Lithium, Prolactin, Protein and Hemoglobin Electrophoresis, Renin, Rubella, VMA, and Catecholamines are other tests frequently referred. There are many tests that are even special procedures for reference laboratories.

The very small laboratories often serve the physician as a "drawing station." These laboratories draw blood from the patient and send everything (including CBCs and differential slides and all serological and chemistry procedures) to the reference laboratories.